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CANCER RESEARCH

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Lipids of the Carcass, Blood Plasma, and Adrenals of the Rat in Cancer*

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The presence of actively growing Walker carcinoma 256 in rats has been shown to affect various non-lipid components of the organs of the tumor-bearing host (1) (2). The present work was undertaken to investigate quantitative changes in carcass lipids and in lipids and steroids in the blood plasma and adrenals of rats bearing Walker carcinoma 256.

MATERIALS AND METHODS

Small pieces of Walker 256 tumor were transplanted subcutaneously into the groin of young male rats. When the tumors comprised from 11 to 48 per cent of the total body weight the animals were sacrificed, tissues removed and the lipids of carcass, blood plasma, and adrenals determined. Normal rats and rats in which the tumor did not grow after transplantation (non-take rats) served as controls.

Rats to be used for determination of carcass lipids were on a diet containing 21.3 per cent fat (3) consisting of coconut oil or cod-liver oil. The remainder of the animals were on a diet of Purina fox chow.

Carcass lipids.—The rats were weighed and killed by decapitation; the tumors were removed and weighed and the carcasses placed in a 30 per cent solution of potassium hydroxide in 95 per cent ethanol. Tumors were dissolved separately in the alcoholic potassium hydroxide solution. After several days the small undissolved residue of bones from each carcass was powdered with a stirring rod and the whole suspension made to 500 ml. with distilled water. Aliquots were evaporated on the steam bath, acidified to Congo red with 3*N* hydrochloric acid and the lipids extracted with a 6:1 mixture of petroleum ether-chloroform. The lipid extract was washed once with 40 per cent ethanol, after which the extract was drawn off into a small weighed Erlenmeyer flask, the solvent removed, and the lipid residue dried to constant weight. The lipids of the tumors were extracted and treated in the same manner.

Plasma lipids.—Blood obtained by heart puncture under ether anesthesia was citrated and centrifuged. To the plasma samples (2 to 4 ml.) were added distilled water to make 4 ml. and sufficient potassium hydroxide to make the solution approximately 8 per cent. The contents of the tubes were mixed and the tubes left on the steam bath overnight (temperature about 85° C.). The solution was then acidified with 10*N* sulphuric acid and extracted for 5 minutes with 3 to 4 volumes of 7:1 petroleum ether-chloroform by use of a shaking machine. The tubes were centrifuged and the lipid extract drawn off. The extraction process was repeated twice after which the extracts were combined and made to a volume of 25 ml. Analysis for total lipid was carried out by a method previously described (4).

Adrenal lipids.—Adrenals were removed from the rats, carefully freed from adhering fat and connective tissue, weighed to the nearest 0.5 mg. and placed in 15 ml. centrifuge tubes. To the tubes were added 4 ml. of distilled water and sufficient potassium hydroxide to make the solution approxi-

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mately 8 per cent. The adrenal suspension was stirred from time to time to dissolve the tissue after which the tubes were left on the steam bath overnight (temperature about 85° C.). The alkaline digests of the adrenals were then extracted with the petroleum ether-chloroform solvent as described under "blood lipids" and the combined extracts made to a volume of 25 ml. This solution of lipids which will be referred to as the "alkaline extract" was found to contain about 85 per cent of the steroid (Liebermann-Burchard reaction).

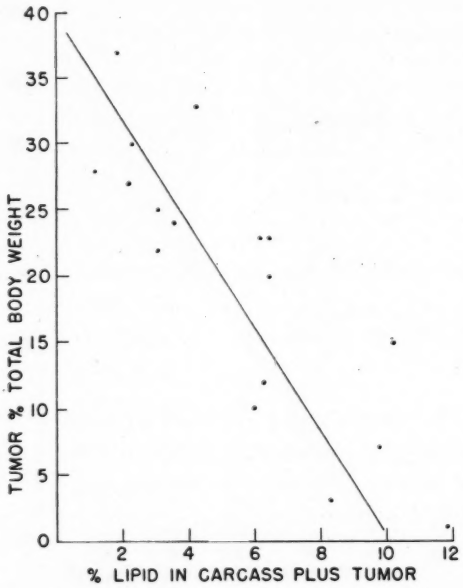

Fig. 1.—The per cent lipid (gm./100 gm. wet weight) in carcass plus tumor plotted against the tumor percentage of total body weight. The average per cent lipid was 9.1±1.7 in the carcasses of 14 "non-take" rats.

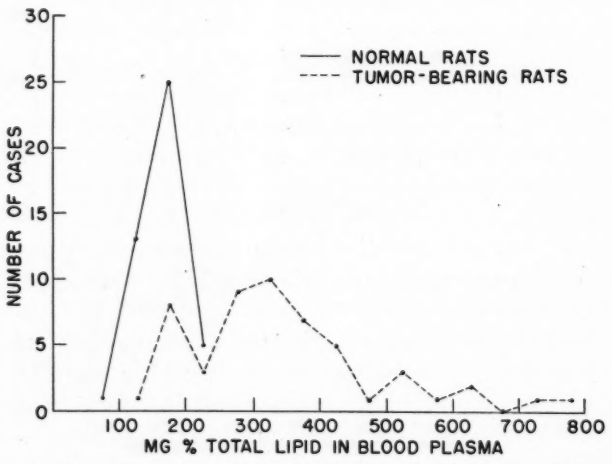

Fig. 2.—Frequency distribution curves showing total lipid in the blood plasma (mg./100 ml.) of normal and tumor-bearing rats.

The residual alkaline water solution was acidified with 10*N* sulphuric acid, and extraction with the petroleum ether-chloroform solvent was carried out as described above. This "acid extract" contained mainly the fatty acids from the glycerides including lecithin and cephalin. The steroid esters were not appreciably hydrolyzed by the alkali and therefore appeared in the "alkaline extract." These extracts were analyzed for total lipid and cholesterol by the method previously published (4).

RESULTS

Carcass lipids.—In Figure 1 the per cent lipid in the combined carcass and tumor of 17 tumor-bearing rats is plotted against the tumor as per cent of the total body weight. The per cent lipid varied inversely with the size of the tumor and was independent of the kind of fat in the diet. Since the average per cent lipid was 9.1 with an average deviation of ±1.7 in the carcasses of 14 "non-take" rats on the same diets, the presence of actively growing tumor decreased the amount of total lipid in the animal.

Total lipid of blood plasma.—Total lipid values on samples of plasma from 44 normal rats averaged 161 mg. per cent with a median value of 166 mg. per cent; range 60 to 245. Values for total lipid of plasma of 52 rats bearing tumors were elevated above normal in most instances. Because of the wide range in values (143 to 790 mg. per cent) and the great variation in size of the tumors (13 to 48 per cent of total body weight) an average would be meaningless. The median value was 325 mg. per cent. Frequency distribution curves for total lipid values of plasma of the normal and tumor-bearing group are shown in Figure 2. It can be seen that the frequency distribution curve for the rats without tumor is essentially normal while that for the tumor-bearing rats is irregularly displaced in the direction of values for total lipid higher than normal.

Cholesterol of blood plasma.—Values for cholesterol of plasma of normal rats averaged 60 mg. per cent with a range of 18 to 84 and a median value of 62. The range in values for plasma cholesterol in the tumor-bearing group was greater (31 to 156) but the median value of 66 mg. per cent was close to the normal. Therefore the elevated values for total lipid of plasma in rats with tumors presumably is due to increased values for total fatty acid of the blood plasma or possibly to increases in steroids other than cholesterol.

Lipids of the adrenals.—The lipid content of the adrenals in normal rats and in tumor-bearing rats grouped according to tumor percentage of the total body weight is shown in Table 1. In rats with large, actively growing tumors constituting over

30 per cent of the total body weight the average per cent total steroids in the adrenals decreased to about one-third of the normal value of 5.3 per cent. In animals with smaller tumors the total steroids of the adrenals decreased but to a lesser extent.

The average values for cholesterol of the adrenals were below normal in all tumor-bearing groups. The decrease in this steroid varied directly with the tumor percentage of the total body weight.

Values for fatty acids had decreased slightly in the adrenals of rats with large tumors but had increased in the adrenals of rats with smaller tumors.

DISCUSSION

The median values for total lipid of the blood plasma in the four classes of tumor-bearing rats showed an increase as the tumor percentage of total body weight increased. In all probability no great significance can be attached to this apparent relationship because of the wide range of values in each group. The increase in plasma lipids in rats with tumors is probably due to increased mobilization from the fat stores which have been greatly depleted in rats with large tumors (Fig. 1). The observation by Mider (5) that rats growing Walker carcinoma 256 lose significantly more total lipid than their pair-fed controls indicates a greater utilization of fat for energy in tumor-bearing than in normal rats. The effect of the various hormones especially those from the pituitary gland on the mobilization of fat is considerable and the possibility of a hormonal explanation for the lipemia must be kept in mind.

By the use of histologic techniques Dalton and Peters (6) observed a decrease in stainable lipid in the adrenals of tumor-bearing mice. Aoki (7) reported a chemical decrease in the concentration of fatty acids, cholesterol, cholesterol esters, and phospholipids in the adrenals of rats bearing a hepatoma.

In our rats growing Walker tumor 256, depletion of adrenal steroids was characteristic of the animals with large tumors and may be explained in the same way as the alarm reaction of Selye (8) or the exhaustion discussed by Long (9). The decrease in the specific steroid, cholesterol, is in line with the current belief that cholesterol is a precursor of the adrenal steroids (8). An inverse relation between the steroid and fat content of the adrenals appears to exist. As the steroids were depleted their place was at first taken by fat and as the animals became more emaciated the fat was used up.

Hyperplasia of the adrenals was also characteristic of the animals with large tumors. The adrenals in these animals sometimes weighed as much as three times the normal. Such enlargement may be interpreted as an effort on the part of the organism to compensate for the deficiency created by the exhaustion. The result is that the amount of steroid per 100 gm. of rat plus tumor was often normal although the percentage content of the adrenal was much below the normal average.

TABLE 1

LIPID CONTENT OF THE ADRENALS IN NORMAL AND TUMOR-BEARING RATS
(GM./100 GM. ADRENAL)

	Total steroids			Cholesterol			Fatty acids			Total lipid		
Rat group	No. rats	Av.	Av. dev.	No. rats	Av.	Av. dev.	No rats	Av.	Av. dev.	No. rats	Av.	Av. dev.
Normal	50	5.3	±1.2	49	2.7	±0.9	50	7.5	±2.1	50	12.9	±2.3
Tumor: per cent of total body wt.												
10–19	28	3.1	1.0	24	1.7	1.0	25	9.8	3.0	25	12.8	3.1
20–29	20	2.1	0.7	19	1.2	0.7	20	9.6	2.6	20	11.9	2.7
30–39	12	1.5	0.5	12	1.0	0.3	11	6.9	2.4	11	8.7	2.3
40–48	14	1.9	0.8	14	1.2	0.6	13	5.1	2.2	13	6.9	1.7

In both adrenals and blood, the range of lipid values was great which was probably due to individual variations in the response of the animals to the presence and growth of the tumors.

SUMMARY

In rats bearing Walker carcinoma 256:

1. Carcass lipids varied inversely with the size of the tumor.

2. Blood lipids, chiefly fatty acids, were increased in most cases; values several times normal were frequently found.

3. In the adrenals: (a) Hyperplasia was characteristic of the animals with large tumors; adrenals frequently weighed as much as three times normal. (b) The average per cent total steroid decreased to about one-third of the normal value in rats with large tumors. (c) The steroids were replaced to some extent by fat.

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The Serum Polysaccharide Level in Malignancy and in Other Pathological Conditions*

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A number of investigators have noted in malignancy a rise of the polysaccharide associated with serum proteins (1, 2, 3, 4, 6, 10). It has also been observed that the serum polysaccharide level tends to be elevated in several other conditions, including hepatic cirrhosis, nephrosis, tuberculosis, pneumonia, and some other febrile conditions. It is the purpose of this paper to present the results of a more extensive survey of the serum polysaccharides in malignancy. In order to furnish data suitable for comparison, a number of other pathological conditions were also studied.

EXPERIMENTAL

Non-glucosamine polysaccharide was determined by the tryptophane method previously described (7), and glucosamine by a modified Elson and Morgan method (8). Total protein was determined by the biuret method according to the procedure of Weichselbaum (9).

Patients for study were selected from those admitted to one of the University Hospitals. Subjects in each group were carefully screened for the absence of other complicating conditions. For the studies on malignancy most of the samples were from patients having a tentative diagnosis before biopsy samples were taken, and before any treatment was initiated. Final diagnosis of malignancy was established in most cases by biopsy; in several instances the diagnosis of carcinoma was based upon x-ray studies and clinical findings.

RESULTS

The findings of 105 patients with malignant conditions are summarized in Table 1. The group included carcinomas of skin (3 basal cell, 5 squamous cell); lung, 9; stomach, 9; pancreas, 6; rectum, 7; breast, 6; cervix, 13; uterus, 4; prostate, 3; bladder, 2; osteogenic sarcoma, 2; chrondrosarcoma, 1; lymphosarcoma, 2; myelogenous leukemia, 7; lymphatic leukemia, 5; monocytic leukemia, 1; Hodgkin's disease, 3; lymphoblastoma, type undetermined, 4; multiple myeloma, 6; and malignant melanoma, 2. These data were compared statistically with data previously obtained for normal adults (8) by the conventional t-test.

The average non-glucosamine polysaccharide and glucosamine levels of serum were significantly elevated, both on a relative and an absolute basis, since the total polysaccharide, expressed either as mg. per cent or as a percentage of serum protein, was significantly elevated. The average serum protein content was significantly subnormal in malignancies. The non-glucosamine polysaccharide/glucosamine ratio was slightly elevated in malignancy but the difference was found not to be significant. The group was subdivided into patients having carcinoma and those with other malignancies. Average results for these groups did not differ significantly, although considerable individual variation was encountered. A few patients, including some with skin cancer or cancer of the breast, exhibited serum polysaccharide values within the normal range.

The serum polysaccharide level of patients with benign lesions was normal in most instances. All of the lesions represented were originally tentatively considered to be malignant before biopsy. The serum polysaccharide was elevated markedly in one patient having hydatiform mole with a non-glucosamine polysaccharide of 203 mg. per cent.

A summary of the results obtained in the study of the serum of 70 non-neoplastic pathological conditions are also listed in Table 1. On the basis of the data, serum polysaccharide levels were elevated in some cases of rheumatoid arthritis, cholelithiasis, ulcerative colitis, acute and nephrotic nephritis, pemphigus, and prostatic hyperplasia. In infections the increase in serum polysaccharide appeared to vary considerably during the course of the disease. It should be noted that the above con-

* This work was supported by a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council, and by funds from the John Archer Hatchett Memorial Fund.

ditions involve inflammation. However, one case of nutritional anemia under treatment, and one of aplastic anemia, also showed elevations of serum polysaccharide. The serum polysaccharide level was essentially normal in all cases of sickle cell anemia, pernicious anemia, allergic bronchitis, atherosclerosis, cirrhosis of the liver, dermatomyositis, diabetes insipidus, diabetes mellitus, epidermolysis bullosa, hyperthyroidism, hypothyroidism, hypertension, congenital hemolytic jaundice, lupus erythematosis, azotemic nephritis, pituitary insufficiency, multiple sclerosis, and thrombophlebitis.

TABLE 1

SUMMARY OF THE SERUM POLYSACCHARIDE LEVELS IN MALIGNANCY AND OTHER PATHOLOGICAL CONDITIONS

	No. of cases	Average	Range	Coefficient of variation*
Non-Glucosamine Polysaccharide mg. %				
(1) Normal adults	43	111	93–127	0.084
(2) Malignancies	105	171	106–308	0.190
(3) Benign lesions	31	123	98–150	0.117
(4) Non-neoplastic pathology	70	149	74–237	0.195
Glucosamine mg. %				
(1) Normals	43	69	61– 82	0.075
(2) Malignancies	105	95	65–177	0.185
(3) Benign lesions	31	76	65– 92	0.084
(4) Non-neoplastic pathology	70	89	50–126	0.203
Non-Glucosamine Polysaccharide÷Serum Protein×100				
(1) Normals	43	1.59	1.26–2.02	0.104
(2) Malignancies	105	2.69	1.76–3.88	0.178
(3) Benign lesions	31	1.96	1.44–2.15	0.102
(4) Non-neoplastic pathology	70	2.41	1.59–5.45	0.322

* Coefficient of Variation = Standard deviation divided by the mean.

TABLE 2

NON-GLUCOSAMINE POLYSACCHARIDE LEVELS OF PATIENTS WITH CARCINOMA OF THE CERVIX

Clinical grade	Number	Non-glucosamine Polysaccharide Av. mg. %	Non-glucosamine Polysaccharide Av. % of Protein
I	3	154(142–163)	2.33(2.19–2.49)
II	5	157(125–187)	2.52(2.38–2.74)
III	3	158(153–162)	2.52(2.25–2.92)
IV	2	248(233, 264)	4.20(3.88, 4.41)

Findings in different types of malignancy.—As previously noted, the serum polysaccharide levels varied somewhat in different types of carcinoma. Patients having carcinoma of the skin, either squamous cell or basal cell, and patients with breast carcinoma tended to have lower values than those in which the carcinoma occurred at other sites. One possible explanation for this difference is that the former are more readily seen and diagnosed, and consequently the lesions were not as extensive when the samples were taken. Unfortunately, data on the size or extent of the lesions were not always available. An attempt to correlate the polysaccharide level with the clinical grade of carcinoma of the cervix is shown in Table 2. It is possible that more careful measurements might reveal a better correlation between the polysaccharide level and the size of the cancerous lesion. Another possibility is that the serum polysaccharide level is affected by differences in the metabolism of various tumors; in this respect, lower levels were found for patients with basal cell carcinoma of the skin as compared with those with the squamous cell type.

The results obtained with leukemia patients were quite variable. Some patients exhibited normal serum polysaccharide and others marked elevation, suggesting that the level may vary during the course of the disease. One patient suffering from chronic lymphatic leukemia, with an initially low serum polysaccharide level, was subjected to serial analyses; but no correlation was found between the polysaccharide level and the white cell count. The polysaccharide level became elevated in terminal stages of the disease without signs of concurrent infection. However, at this stage a high icteric index and a 4+ cephalin flocculation were obtained, indicating possible hepatic complications.

Exceedingly high values for serum polysaccharide were found in cases of multiple myeloma. Because of the accompanying hyperproteinemia in these cases, the relative values were somewhat lower than the average for malignancy.

The "TA" factor and serum polysaccharides.—Seibert, Pfaff, and Seibert (5) have recently described an arbitrary method for the determination of a serum fraction which is apparently polysaccharide in nature. They suggest that the test might have some value in the diagnosis of carcinoma as well as of tuberculosis. The test was used in this laboratory essentially as originally described. Since variations were encountered with the same sample of serum when determinations were made at different times, a standard serum was used in each set of analyses and the results calculated by relationship to it. As the original color of the sera seemed to have little relationship to the color resulting from the reaction, a correction for the original color of the serum was omitted. The colorimetric analysis was made on a Coleman 11 spectrophotometer, and the results were expressed simply as optical density. Of the 70 subjects studied, 5 were normal, 36 had malignancies, and the remainder included nephrosis, tuberculosis, tularemia, tonsillitis, pregnancy, hemolytic jaundice, and

benign neoplasia. In general, the TA results varied with the non-glucosamine polysaccharide, the correlation coefficient for the 32 cases of malignancy being 0.83, a highly significant figure for this number of cases. However, in conditions in which jaundice occurred, the TA results were much higher than the corresponding polysaccharide value. Neither a correction for the original color of the serum nor a correction made by following the procedure without the addition of tryptophane corrected these high values. Inspection of the results did not indicate that the TA reaction is any more sensitive to the presence of cancer than is the polysaccharide determination. The TA value was elevated in all non-malignant cases in which the polysaccharide was elevated.

Serum polysaccharide level as a diagnostic aid.—In order to evaluate the use of the non-glucosamine polysaccharide level in the diagnosis of malignancy, the data on 105 patients with malignancies and 32 patients with benign lesions are grouped in Table 3. Although the data on benign lesions is too meager for exact conclusions, the grouping indicates that if the serum polysaccharide level were used to distinguish between benign and malignant lesions a number of false positives would be obtained at any level. The most successful criteria would be at either a non-glucosamine polysaccharide level above 140 mg. per cent or a ratio of polysaccharide to protein of 2.20 per cent, in which instance 90 per cent of the malignancies and 9 per cent of the benign lesions in this study would give positive results.

If only cancers of the lung, stomach, pancreas, rectum, cervix, uterus, prostate, and bladder are considered, 52 out of 53 cases (98 per cent) exhibited serum polysaccharide levels of over 140 mg. per cent or ratios over 2.20. It is in this type of neoplasia that a diagnostic method would be of considerable value. In order to use the serum polysaccharide level for assistance in the diagnosis of malignancy, other conditions in which elevation occurs would have to be ruled out. Since the level seems to be elevated invariably in inflammatory processes, it might serve as a diagnostic screening test for the presence of such a process. In any case, closer correlation between chemical and clinical studies is indicated before drawing conclusions regarding the possible clinical use of this test. Further investigation may reveal an elevation of polysaccharide associated with some particular serum protein fraction or fractions that may be more characteristic of malignancy. Studies of this possibility will be reported in a later paper.

Significance of the serum polysaccharide elevation in malignancy.—In order to properly evaluate the elevation of serum polysaccharide in malignancy, it is important to establish the cause of this elevation, which might be a secondary effect. Seibert *et al.* (6) suggest that the elevation is attributable largely to an increase in α_2 globulin, as this fraction normally has a high polysaccharide content and it tends to increase in diseases involving tissue destruction. In a later paper (5) these investigators were unable to account for the actual polysaccharide levels of sera from patients with cancer and late tuberculosis by calculations based on electrophoretic analysis and determination of polysaccharides in fractions of normal plasma proteins. However, they found good agreement between actual and calculated values in fetal and normal sera, and for patients with active minimal tuberculosis and sarcoidosis. The discrepancy might be interpreted as indicating the abnormal appearance in carcinoma and tuberculosis of polysaccharides absent from or present in small amounts in normal sera.

It is difficult to explain the elevations encountered in prostatic hyperplasia on the basis of tissue destruction. A more reasonable correlation might

TABLE 3

DISTRIBUTION OF PATIENTS WITH MALIGNANT AND BENIGN LESIONS ACCORDING TO NON-GLUCOSAMINE POLYSACCHARIDE LEVEL

Group	Malignant Cases		Benign Cases	
	No.	Percentage	No.	Percentage
Polysaccharide level above 130 mg.%	96	92	7	22
" " " 140 mg.%	90	86	4	12
" " " 150 mg.%	82	78	1	3
Polysaccharide as % of Protein above 2.02%	100	95	9	28
" " " " " 2.10%	94	90	7	22
" " " " " 2.20%	89	85	1	3
Either above 130 mg.% or above 2.02% of protein	101	96	9	28
" " " mg.% " " 2.10% " "	98	93	8	25
" " " mg.% " " 2.20% " "	97	92	7	22
" " 140 mg.% " " 2.10% " "	98	93	8	25
" " " mg.% " " 2.20% " "	95	90	3	9
" " 150 mg.% " " 2.20% " "	92	88	1	3

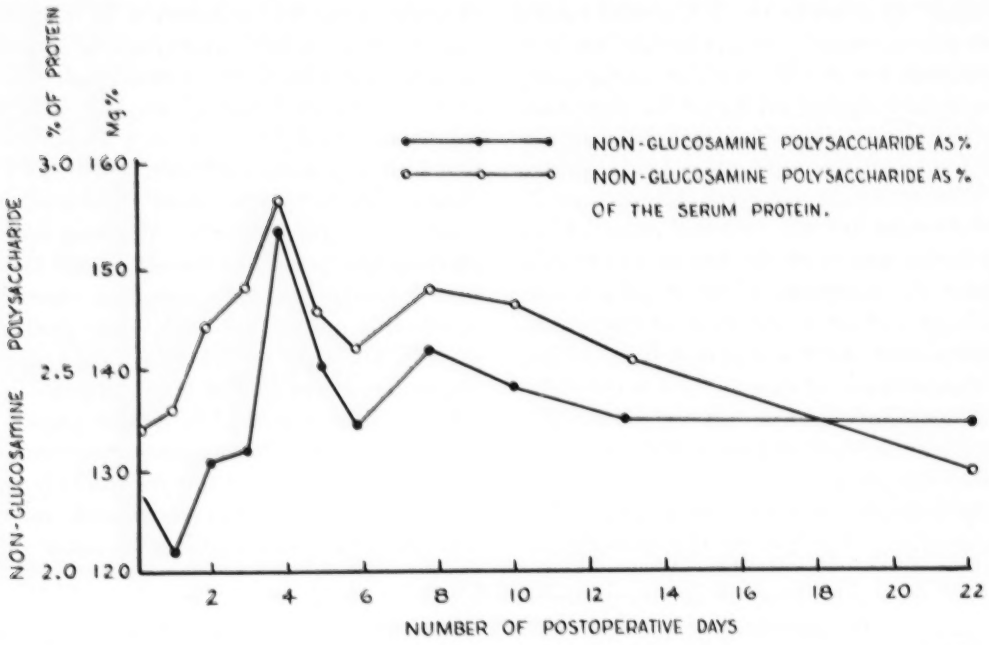

FIG. 1.—The serum non-glucosamine polysaccharide level of a dog—following an experimental surgical operation.

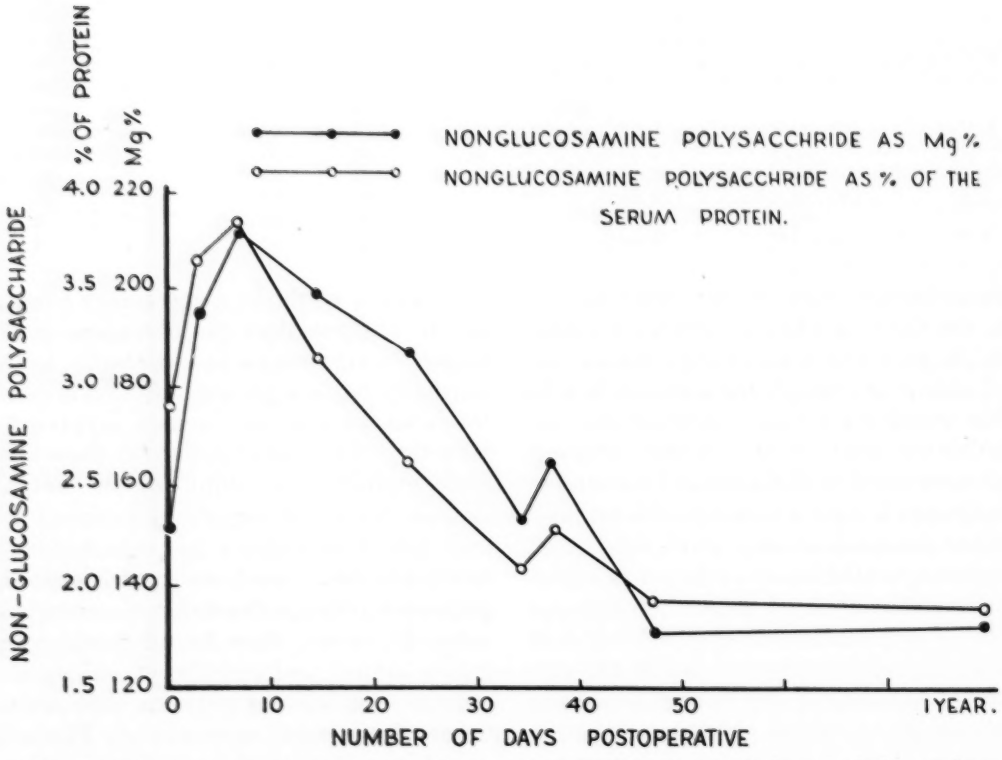

FIG. 2.—The serum non-glucosamine polysaccharide level following surgical removal of a carcinoma of the cervix. Deep x-ray therapy started on the 32d day.

be with tissue proliferation. The rise which occurs in inflammation could also be connected with tissue repair rather than with tissue destruction. Data obtained by making serial studies during an experimental operation are presented in Figure 1.

This operation was performed on a female dog weighing 5.5. kg. A 10 cm. incision was made in the peritoneal wall and the ovaries and part of the uterus were removed. The operation was carried out under aseptic conditions. The wound was carefully closed in layers and bandaged. Daily injections of 50,000 units of penicillin were given for 5 days to reduce the dangers of infection. No temperature elevation was noted during the post operative course. As shown in Figure 1 the maximum serum polysaccharide level expressed as a percentage of the serum protein occurred on the fourth day. The elevation was slight during the first 24 hours when the effects of tissue injury should preponderate. The absolute values of the serum polysaccharide actually decreased during this period, but this effect may be ascribed to the decrease in serum protein which occurred during the same interval. Further studies concerning the effects of inflammation on serum polysaccharides will be reported in a later communication.

Effect of surgical removal of a neoplasm.—Data obtained by serial serum studies of a patient from whom a carcinoma of the cervix was surgically removed is shown in Figure 2. Again the post-surgical elevation of serum polysaccharides reached its maximum in several days. The level then fell gradually and approached normal a month after the operation. A subsequent small temporary rise following x-ray treatments might be attributed to inflammation. Similar elevations have been noted in other cases of malignancy where x-ray therapy was used. This patient has now been followed for 18 months and has exhibited no clinical signs of recurrence. The fact that the polysaccharide level has remained essentially unchanged since the second post-operative month suggests that the determination might have some value as a prognostic aid in certain types of cancer.

SUMMARY AND CONCLUSIONS

Malignancy was found to cause a significant elevation of the serum polysaccharide level in a series of 105 cancer patients, 96 per cent having levels above the highest normal level. The polysaccharide levels associated with cases of skin and breast carcinoma were somewhat lower than in other types of malignancy; no difference was noted between carcinoma patients and those having other types of malignancy.

Patients with benign lesions exhibited serum polysaccharide levels which were normal or only slightly elevated. A very high level was found for one patient with hydatiform mole. Of other pathologic conditions studied, arthritis, cholelithiasis, ulcerative colitis, nephritis, pemphigus, and most infections caused elevations of serum polysaccharide. Four out of six patients with prostatic hyperplasia exhibited a level higher than normal.

Following experimental operations on dogs, the serum polysaccharide rose to a maximum in 4 days. A similar rise with maximum elevation on the seventh day occurred after operation for the removal of a carcinoma. Subsequently the serum polysaccharide decreased and became normal by the forty-eighth day. Treatment of this patient with x-ray post-operatively caused a temporary rise in the polysaccharide level. The suggestion is made that elevations of serum polysaccharide are involved in some way with tissue proliferation.

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Effect of Tubercle Bacilli Extracts on Induced Tumors of the Rat

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Nauts, Swift, and Coley (14) gave an exhaustive review of the clinical reports, and of animal experimentation, on the influence of bacterial toxins on malignant tumors, since the method was first used by W. B. Coley in 1892.

Beebe and Tracy (2) found that suspensions of *Bacillus prodigiosus*, *Streptococcus pyogenes*, *Staphylococcus aureus*, and *B. coli communis* caused regression of large transplanted lymphosarcomas in the dog. Similar results were obtained with bacterial preparations by Uhlenhuth, Haendel, and Steffenhagen (27) on rat tumors. Positive results were obtained only when the material was injected directly into the tumor. Gratia and Linz (11) found that *B. coli* filtrates produced hemorrhage and liquefaction in transplanted liposarcomas in the guinea pig.

Schwartzman and Michailovsky (21) reported the complete disappearance of tumors in mice after injecting meningococcus filtrate. Duran-Reynals (8) used *Eberthella typhosus*, *B. enteriditis* and *B. paratyphosus* filtrates and showed that in mice the newly formed tumor capillaries were very sensitive to these toxins. Apitz (1) studied the hemorrhagic reaction in 197 mouse carcinoma by means of *B. coli* filtrates and other bacterial substances and concluded that the walls of the tumor capillaries were damaged. This was not a generalized vascular reaction, but was confined to the tumor capillaries.

Shear began in 1933 to fractionate the hemorrhage-producing substance from *B. prodigiosus* and found it to be a polysaccharide. Following this he (22) studied the effect of meningococcus filtrate and various bacterial metabolites and found that in certain cases liquefaction and hemorrhage of the tumor occurred with an occasional complete regression. He (23) next separated the hemorrhage-producing fraction of the *B. coli* filtrate and produced extensive hemorrhage in mouse tumors. Severe hemorrhage was produced in subcutaneous primary induced mouse tumors by Shear (24) within a few hours after injecting the concentrated filtrate from *B. prodigiosus* in these tumors, but not in the normal tissue.

Shear (25) used doses as low as part of a microgram from the polysaccharide of *B. prodigiosus* on 750 mice bearing subcutaneous primary tumors, induced by 3,4 benzypyrene, and produced hemorrhage in them within a few hours. Diller (6) found that the polysaccharide of *B. prodigiosus*, when injected intravenously into a tumor-bearing animal, caused nuclear changes in the tumor cells. These changes involved surface blistering, shrinkage, pyknosis, and attenuation of nuclei leading ultimately to their complete destruction.

Of special bearing on our problem are the extensive studies by Morse and Scott (13) on histological changes produced in normal animals by the lipoid, protein, and polysaccharide fractions of the tubercle bacillus. Sabin and Doan (16) observed numerous cellular changes in rabbits after injecting the proteins and phosphatide fractions of human tubercle bacilli and in 1930 Sabin, Doan, and Forkner (17) reported further studies on the reaction of normal rabbit tissues to lipoid, protein, and polysaccharide fractions of *Mycobacterium tuberculosum H37*. Sabin *et al.* (18) found that the polysaccharide may have some killing power under certain conditions, but this is not as consistently related to dosage as in the case of proteins. Smithburn and Sabin (26) showed that the phosphatide fraction stimulates the formation of characteristic epithelioid and giant cells. Schaefer (20) separated the lipoid, polysaccharide, and protein fractions of tubercle bacilli and studied their action on normal mammalian tissues. Delaunay *et al.* (5) have demonstrated the leucocytic chemotactic power of certain constituents of tubercle bacilli in the guinea pig.

It is the purpose of the writers to describe the effect of the protein, phospho-lipoid, and polysaccharide fractions of *Mycobacterium tuberculosum* on methylcholanthrene-induced tumors in the rat.

MATERIALS AND METHODS

The tumors were induced by a single injection of 10 mg. of methylcholanthrene (Eastman Kodak Co., Rochester) dissolved in sterile olive oil. The rats were

injected subcutaneously in the left inguinal region and spindle-celled sarcomas appeared in between 4 and 8 months. Injections of the bacterial filtrates were started as soon as the tumor was visible macroscopically. The sex and weight of the tumor-bearing rats were recorded at the time we started injecting the bacterial extracts, which were made three times weekly. At the same time macroscopic observations of the tumor, the general appearance of the rat, and measurements of the tumor, were recorded.

Preparation of Mycobacterium tuberculosum extracts.—Extracts were made from six to eight weeks old *M. tuberculosum hominis* cultured on the surface of Sauton's synthetic medium at 37° C.

The phospho-lipoid, protein, and polysaccharide fractions were extracted according to the methods described by Schaefer (20).

To extract the phospho-lipoid fraction the cultures were first sterilized at 120° C. for 1 hour, passed through filter paper and washed on the filter with distilled water. The bacilli were then dried in a vacuum jar containing calcium chloride, weighted and treated with pure acetone for 24 to 36 hours at 25° C. (1 cc. acetone to 100 mg. of bacilli). The bacilli were filtered again, dried as before and placed in 99 per cent methyl alcohol for 10 days at 37° C. (1 cc. methyl alcohol per 100 mg. of bacilli). The amount of total phosphate in the filtrate was determined by the method of Fiske and Subbarrow (9). This methyl alcohol filtrate was diluted 1:10 with sterile normal saline (0.85 per cent) containing 0.5 per cent phenol and tubed into sterile vaccine bottles. The final concentration of the filtrate was 0.001 mg. per cc. The filtrate was stored in the refrigerator at 5° C. The yield of phosphates from the bacilli varied between 0.02 to 0.004 mg. per 1 gm. of dried tubercle bacilli, the average of 4 determinations being 0.012 mg.

To extract the protein fraction the living cultures were first passed through filter paper after which the filtrate was passed through a Seitz filter. To each liter of filtrate 600 gm. of ammonium sulfate was added in order to precipitate the proteins and the polysaccharides. After standing one hour the filtrate was centrifuged at 2500 r.p.m. for 20 minutes, the supernatant fluid was discarded, and the precipitate was dissolved in distilled water. In order to remove the ammonium sulfate the filtrate was next placed in a cellophane bag, containing thymol as preservative, and dialyzed against distilled water at 5° C., the water being changed 3 to 4 times daily until free from ammonium, as shown by a negative reaction with Nessler's reagent. The solution was then dried in vacuum over sulfuric acid. The dried material, which included the proteins and polysaccharide fractions of the bacilli, amounted to 0.520 to 0.404 mg. per cc. of the original filtrate; average of four determinations was 0.474 mg. per cc. of filtrate.

The next procedure was the separation of the protein and polysaccharide fractions from one another. The dried mixture was weighed and made into a 2 per cent solution in neutral distilled water. To this was added 40 per cent trichloroacetic acid (20 cc. acid to 100 cc. solution), and the precipitated proteins were separated by centrifugation. The supernatant fluid, which contains the polysaccharide, was decanted and saved. The precipitate, containing the proteins, was extracted with water and reprecipitated with trichloroacetic acid. This process was carried out three times, the supernatant fluid being retained in each case. After the third precipitation the protein precipitate was extracted in neutral distilled water and the extract was dialyzed for 24 hours at 5° C. This fraction was dried in a vacuum, dissolved in sterile saline (0.85 per cent) containing 0.5 per cent phenol, and tubed into sterile vaccine bottles. The final concentration of protein, 1.5 mg. per cc., was stored in the refrigerator at 5° C.

To extract the polysaccharide fraction the combined supernatant fluids obtained, as shown above, were treated with three volumes of 96 per cent ethyl alcohol, plus one volume of ether, in order to precipitate the polysaccharides. The precipitate was obtained by centrifugation and was washed twice with ether, and dried in vacuum over sulfuric acid. The dried polysaccharides were dissolved in sterile saline (0.85 per cent) containing 0.5 per cent phenol. The final concentration of the polysaccharides was 2 mg. per cc. The material was tubed into sterile vaccine bottles and stored in the refrigerator at 5° C.

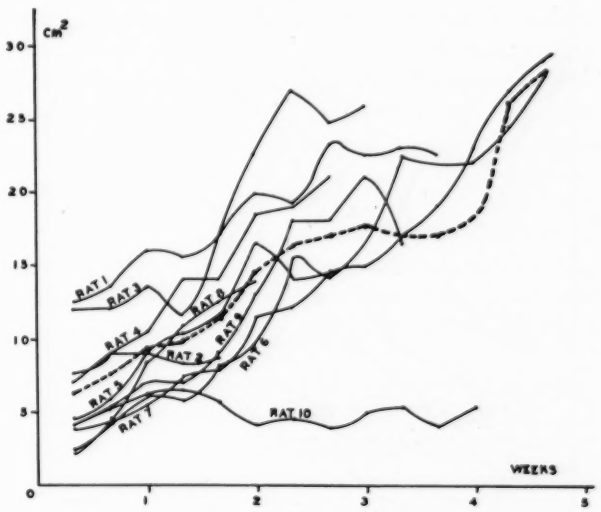


FIG. 1.—Graph showing surface areas, in square centimeters, of tumors treated with the phospho-lipoid fraction. The broken line represents the average. The termination of the individual curves indicates the time of death of the animal.

Method of injection.—Into each of 10 tumor-bearing rats 0.001 mg. of the phospho-lipoid fraction was injected at the site of the tumor 3 times weekly. The minimum number of injections, before the death of the animal, was 5 and the maximum number was 15 (Fig. 1).

Thirteen tumor-bearing rats had 1 mg. of the protein fraction injected 3 times a week into the tumor. Three of the rats died after the second injection and two after the fifth, while 5 had from 8 to 11, and 3 had 14 injections (Fig. 2).

Into 11 tumor-bearing rats 1 mg. of the polysaccharide fraction was injected into the tumor 3 times a week. Two rats died after the seventh injection and the rest had from 9 to 14 injections (Fig. 3).

RESULTS AND OBSERVATIONS ON TUMOR-BEARING RATS

Phospho-lipoid fraction.—Ten rats with tumors were used in this series. On macroscopic observation of the tumor, bleeding was observed in most animals while introducing the needle after the third and on subsequent injections. The tumor became soft, congested, and extensively hemorrhagic. The condition of the animal became gradually worse, the tumor continued to increase in size and most of the rats died within about 4 weeks. Measurements were made of two diameters of the tumors at the time of each injection; however, the depth of the tumor could not be ascertained accurately and was not taken. These dimensions were multiplied to give surface values in square centimeters.

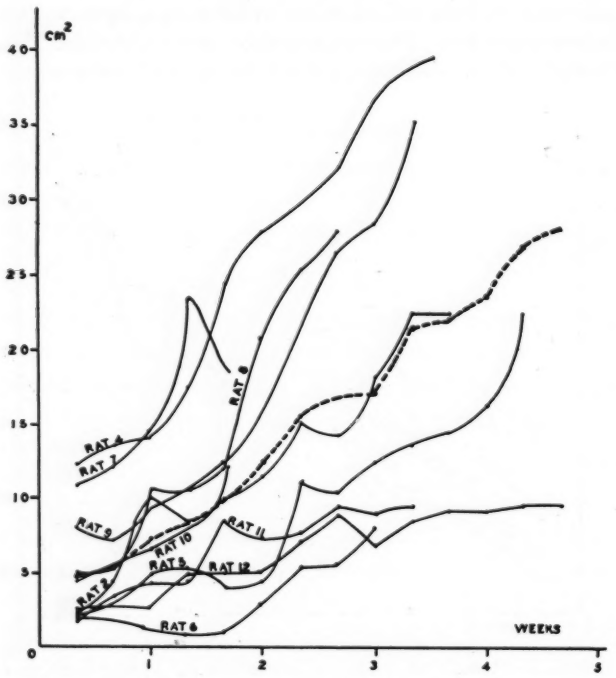


FIG. 2.—Graph showing surface areas, in square centimeters, of tumors treated with the protein fraction.

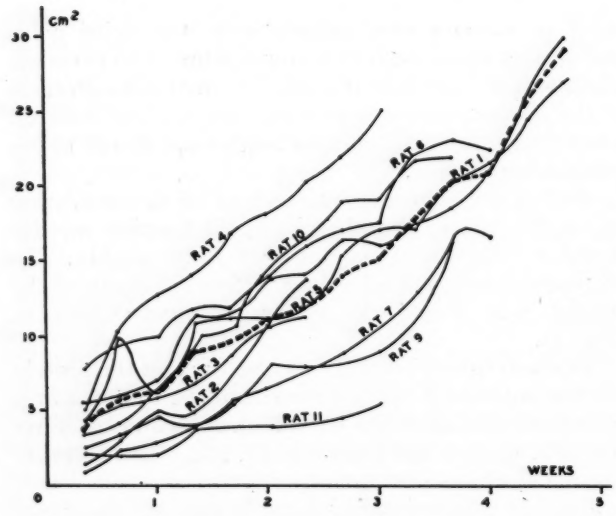


FIG. 3.—Graph showing surface areas, in square centimeters, of tumors treated with the polysaccharide fraction.

Figure 1 shows that the growth of the tumors followed more or less the same pattern with three deviations. Rat No. 10 showed no increase in the size of its tumor, while rats Nos. 1 and 3 had a much higher rate of growth than the average. An "average graph," based on the averages of the ordinates, is represented by the broken line.

Microscopic observations showed no evidence of cell destruction or of tumor regression following the injections that could be ascribed to the fraction (Figs. 4 and 5). There were, however, the usual areas of necrosis characteristic of a rapidly growing spindle cell sarcoma, but there was no lymphocytic infiltration.

Protein fraction.—Thirteen rats were used in this series. As in the case of the phospho-lipoid fraction, profuse hemorrhage was noted. There is more deviation in the tumor sizes of this group than in the preceding. Rats 4, 8, and 9 had much faster growth rates, while rats 6, 11, and 12 were quite sluggish; however, the "average graph" is comparable to the graphs for the other two fractions (Fig. 2).

Microscopically there was no evidence of more cellular destruction than the usual variations in untreated tumors.

Polysaccharide fraction.—In most of the eleven rats of this series, hemorrhage was indicated by softness of the tumor and the appearance of blood following the third or fourth injections.

More uniformity was encountered in the tumor growths of this group of rats. The tumor of rat 11 failed to grow and in this manner resembles rat 10 of group 1, and to a lesser extent rats 11 and 12 of group 2. The "average graph" is similar in shape and extent to the other two (Fig. 3).

The microscopic observations following the polysaccharide fraction were similar to those previously described. All the tumors were highly malignant, with no pattern of arrangement, and many multi-nucleated cells were present. The amount of blood vessels was variable, with areas of hemorrhage and necrosis.

RESULTS AND OBSERVATIONS ON CONTROL RATS

Phospho-lipoid fraction.—Three control rats each received a total of 14 injections of the phospho-lipoid (0.001 mg.) fraction subcutaneously in the left inguinal region. Bleeding was not apparent at any injection, in any of the controls, and all the

animals survived the treatment. After the fourteenth injection the control rats, and some of the tumor-bearing rats, were killed and both the left and right inguinal areas were fixed in formalin, sectioned in paraffin, and stained with hematoxylin and eosin.

Microscopically, distinct tubercles were observed at the site of the injections (Figs. 6 and 7) with typical epithelioid and giant cells. The reaction was in the connective tissues and muscle, but the lymph nodes had no tubercles. There was an intense diffuse mononuclear response and lymphocytes, fibroblasts, and giant cells were observed in the region of the injection.

Protein.—Three rats were given 14 injections (1 mg.) of the protein fraction. Slides made from tissue in the region of the injections show a non-specific foreign body reaction. There was a mononuclear cell infiltration and giant cells; lymphocytes, and phagocytes were also observed.

Polysaccharide.—Three control rats received a total of 14 injections (1 mg.) of this fraction. There were lymphocytes, phagocytes, giant cells, and many fibroblasts, but there was no specific reaction in the injected area. The lymph nodes in the region showed no tubercles, but diffuse mononuclear cells were numerous.

DISCUSSION

In a series of preliminary studies we prepared and injected a series of tumor-bearing rats with Coley's toxin, and the filtrates of *E. typhosus* and *S. paratyphosus*. Our results were comparable to those of Coley (4), Duran-Reynals (7), Shear (25), and others.

Sabin and Doan (16) observed in normal rabbits numerous cellular changes after the injection of protein and phosphatide fractions of the human tubercle bacilli. The phosphatide caused development of monocytes, epithelioid cells, and Langhans giant cells making a typical tubercular lesion. Lymphocytic infiltration and some necrosis were also noted. Their protein fractions caused multiple capillary hemorrhages and an increase in debris and bacilli filled clasmatocytes. Sabin (19) also found that the protein fraction induced the formation of monocytes in the normal animal, and tubercles of epithelial cells in tuberculous animals.

After considering the findings of previous workers, we chose to inject the fractions into the tumors rather than intravenously or intraperitoneally. Beebe and Tracy (2) and Uhlenhuth *et al.* (27) found positive results only when their material was injected into the tumor; furthermore dead tubercle bacilli are toxic when injected intravenously or intraperitoneally.

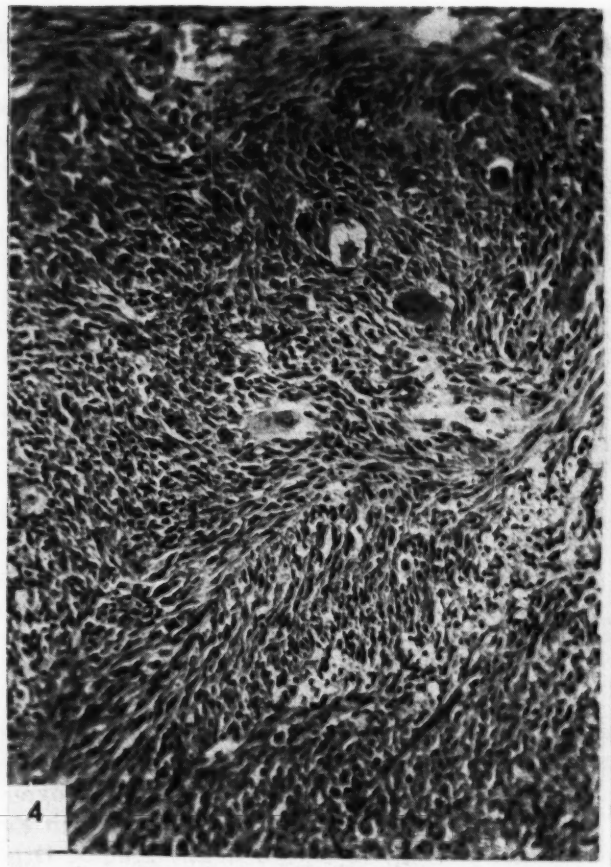
The dosage we used was based on comparable work of previous authors for other bacterial products, but was near the higher rather than the lower dosages. Long and Seibert (12) used 1/10,000 mg. of precipitated protein with ammonium sulfate per guinea pig and Sabin (19) gave 30 mg. of a similar fraction to each rabbit. Our 1 mg. dosage given 3 times a week for a period of 3 to 5 weeks seemed to be a plausible amount for rats.

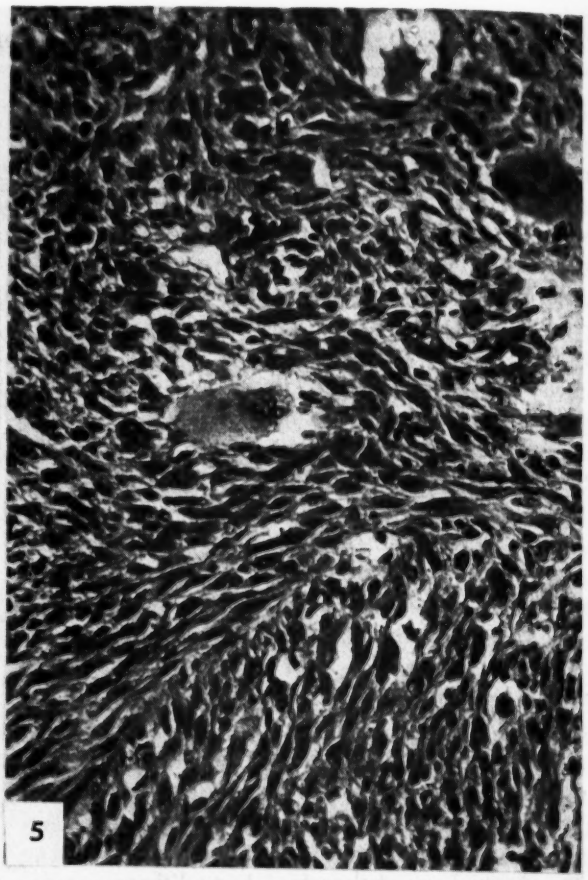
Our macroscopic findings agree fairly well with those of previous workers in the presence of hemorrhagic and softening areas; however, it should be recalled that untreated tumors often have such areas, and that our treated tumors were not affected in a regressive manner. In spite of the hemorrhage the growth curves showed that the tumors continued to grow, more or less, in a uniform pattern and rate, in the three different filtrate series, with a few exceptions which remain unexplained. The exceptional cases were rat No. 1 of the phospho-lipoid series and rat 11 of the polysaccharide series whose tumors did not progress. Some other rats had a much higher growth rate than the average as shown in the graphs. Other than these exceptions the growth curves in the three series were very similar and there is no evidence of one extract being more effective than another.

Histologically we found that control rats, without tumors, showed similar reactions to those described in mice and rabbits by the earlier authors quoted above, but to a less striking extent, due perhaps to the higher resistance of the rat. Difference in resistance was shown by Gerstl and Tennant (10) who found that the mouse is more resistant than the rabbit, or the guinea pig, to extracts from tubercle bacilli and they attribute this to its more rapid enzyme action. Pinner (15) also states that rats are highly resistant to tubercle bacilli and do not develop destructive tissue processes.

We have observed that the phospho-lipoid fraction caused in the normal rat distinct tubercles with typical epithelioid and giant cells, with infiltration of monocytes, lymphocytes, and with some necrotic areas. The polysaccharide fraction produced streaks of white blood cells in addition to other cellular elements at the site of injection in the normal rat. After the protein fraction we found in the normal rat a mononuclear cell infiltration. Giant cells, phagocytes, and lymphocytes were also observed; however it was a non-specific foreign-body reaction.

In our tumor-bearing rats, on the other hand, we failed to note such a tissue reaction as we de-





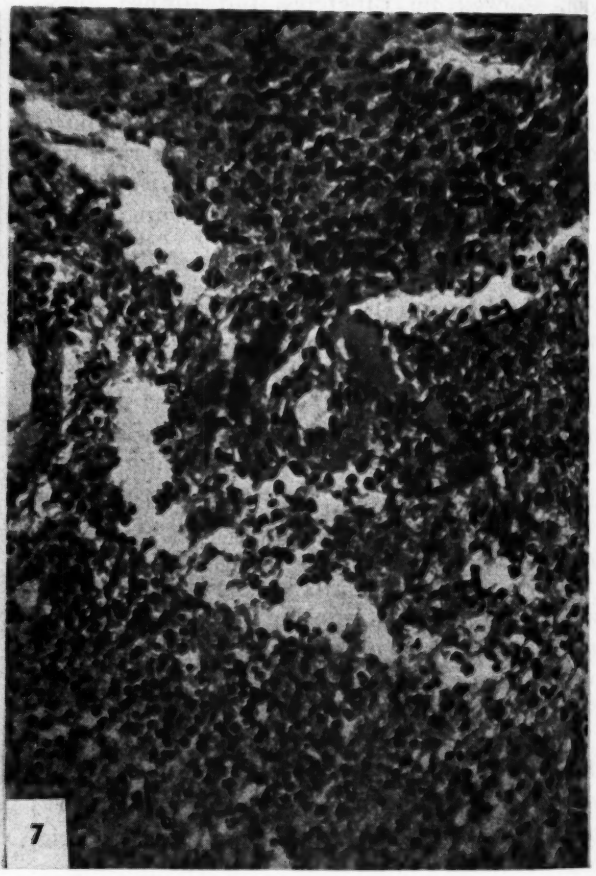


FIG. 4.—Section of tumor treated with phospho-lipoid fraction. Hematoxylin and eosin. ×140.

FIG. 5.—Same as Fig. 2. ×280.

FIG. 6.—Section from the site of injection of phospho-lipoid showing tubercle formation in control rat. Hematoxylin and eosin. ×140.

FIG. 7.—Same as Fig. 4. ×280.

scribed in the normal rats. We found no evidence of destructive changes due to the filtrates, for the hemorrhagic and necrotic areas, such as we found, are usually present in a rapidly growing methylcholanthrene-induced rumor of the same kind, hence cannot be attributed to the filtrates themselves.

In conclusion, the three extracts of the tubercle bacilli used, which were capable of stimulating some tissue reaction in the normal rats, produced no destructive effects on the fibrosarcomas of the same animal. It might be worth while to repeat the work with an animal that is more susceptible to the tubercle bacillus.

SUMMARY

1. Three extracts of the tubercle bacillus: phospho-lipoid, a protein, and polysaccharide were prepared.

2. Tumors were produced in rats by subcutaneous injections of methylcholanthrene.

3. Injections of the extracts were given into the tumors, the growth was measured, and histological studies were made. No evidence of destructive effect of the extract was noted.

4. Control rats were given, in the inguinal region, the same extracts. Histological studies revealed tissue reaction, as expected, with the appearance of typical tubercles with the phospholipoid fraction.

5. It is suggested that a more susceptible animal than the rat should be used for the same work.

ACKNOWLEDGMENTS

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Experimental Production of Endometrial Polyps in the Guinea Pig*

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Under the term endometrial polyps is grouped a variety of tumors of polypoid structure which are attached to the inner uterine wall by pedicles of variable length and thickness. Although their histologic characteristics differ greatly, the most common variety has a tissue structure similar to that of the surrounding endometrium. The polyps may be single or multiple; small, or large enough to occlude the uterine lumen. In the human, sloughing of the tip in the pedunculated variety may be the cause of persistent hemorrhage (1, 2).

Endometrial polyps in laboratory and domestic animals occur rarely and apparently have not been produced experimentally in any animal.

During an investigation on the embryo-endometrial interrelationship at the time of implantation in the guinea pig (3), it was found that endometrial polyps could be produced if small inert objects were implanted just below the epithelium of the endometrium at any time between the second and seventh days after ovulation. Therefore, an investigation was undertaken to determine: (1) the frequency of occurrence of endometrial polyps in the normal guinea pig; (2) the time during the reproductive cycle that polyps could be produced by the techniques to be described; (3) whether polyps could be induced to develop in all regions of the endometrium; and, (4) the ultimate fate of the polyps.

MATERIALS AND METHODS

The data presented here were obtained from 157 sexually mature female guinea pigs. The animals were maintained on a ration of oats, hay, green vegetables, and water. The events in the female reproductive cycle were timed from the onset of the animal's willingness to mate as determined, either by placing the female with a vigorous male at hourly intervals, or the animal's response to manual manipulation as described by Young, Dempsey, and Myers (4). In the experimental animals the endometrial polyps were induced in the following manner: Perfectly round glass or paraffin beads, 50μ to 75μ in diameter, were prepared according to the method described elsewhere (3). The paraffin beads were sterilized before use by repeatedly rinsing them in 70 per cent alcohol and sterile distilled water; the glass beads were boiled in distilled water. A single bead was inserted into the end of a number 27 hypodermic needle which had been ground very short and to a fine point. A stylet was fitted into the bore of the needle so that a single bead could be pushed from it after the end of the needle had been placed in position in the endometrium. Using aseptic technique the cornua were exposed separately by incisions made through the axial muscles of the back. The needle was inserted into the uterus by directing it from the surface toward the lumen as carefully as possible so that there would be only minimal tissue damage. Attempts were made to place the beads just below the epithelium lining the lumen. Because of the blind method of inserting the beads they could not always be placed in the desired position. However, a sufficient number of beads were inserted so that at least some beads were located in all regions of the endometrium from the antimesometrial to the mesometrial areas (Fig. 1). The animals into which beads had been implanted between the second and seventh days were killed for examination between the twelfth and sixteenth days after the onset of heat. Those in which beads were implanted after the seventh day were examined either toward the end of that reproductive cycle or during the succeeding cycle.

In the guinea pig the vaginal orifice becomes occluded by the growth of a thin vaginal membrane several days after the end of heat. The vaginal membrane of each experimental animal was examined daily in order to detect the possible presence of blood behind this membrane, thus indicating the presence of a bleeding polyp.

As controls, sham operations were performed including the insertion of the needle into the uterus but without the deposition of a bead. If at the time of killing, polyps, or subepithelial beads which had not induced polyps were found, the tissues were removed, fixed in Bouin's fluid and prepared for histologic examination in the usual manner.

* This study was aided by grant CT583 from the National Advisory Cancer Council of the United States Public Health Service.

OBSERVATIONS

Frequency of occurrence of endometrial polyps in the normal guinea pig.—Small endometrial polyps were observed in only 3 per cent of 200 cornua of normally mated and unmated females in all stages of the reproductive cycle. This survey was made in conjunction with another investigation in which the entire luminal surface of the endometrium was carefully examined with a binocular dissecting microscope (5). The polypoid outgrowths found in these normal animals ranged in length from 1 to 3 mm. One such polyp is shown in Figure 2. It appeared as a small outgrowth from the region of the antimesometrium and was attached to it by a rather broad base. The histologic characteristics of these polyps vary. Some were composed of a connective tissue core completely covered by either a simple or a stratified layer of epithelium. In general they contained the same histologic components as that of the underlying endometrial stroma (Fig 3). Several of the polyps were devoid of a covering epithelium and were composed of cells which appeared similar to those of the subepithelial stroma. Even though the number of polyps observed in normal animals is small there is no significant difference in the frequency of their occurrence in pregnant and non-pregnant animals. From the observations made it may be concluded that endometrial polyps in the normal guinea pig occur rarely and do not grow to a large size.

Experimentally induced polyps.—The site of formation in the cornua and the time in the reproductive cycle that endometrial polyps could be produced was investigated in 57 animals. It was observed that polyps could be induced to develop, by the method described, between the second and seventh days after the onset of heat. A total of 35 endometrial polyps was observed in 33 of the 57 cornua into which beads had been inserted. Polyps could be produced most consistently from beads implanted near the apex of the antimesometrium on the fifth and sixth days after ovulation. No polyps developed from beads implanted between the eighth day and the onset of the succeeding heat period.

As mentioned earlier the control experiment consisted of sham operations on the opposite cornu into which the needle used for implanting the beads, was inserted through the uterine wall in the same manner as if a bead were to be deposited. In no instance did a polyp develop as the result of this procedure.

Typical examples of the experimentally induced endometrial polyps in both the fresh and fixed condition are shown in Figures 4 through 8. All of these polyps were removed from females in which beads had been implanted on either the fifth or sixth day and the animals killed on the twelfth day of the same cycle. The majority of polyps were attached to the endometrium by a narrow base and invariably originated within the area of the apex of the antimesometrium. The rate of development of the polyps varied considerably. It is possible that the differences in size were dependent upon the extent of stimulation on the endometrium at the time the bead was inserted. In the majority of cases the distal end of the polyp was bulbous in appearance (Figs. 4 and 6) although occasionally the body of the polyp was wider than its distal end (Fig. 8).

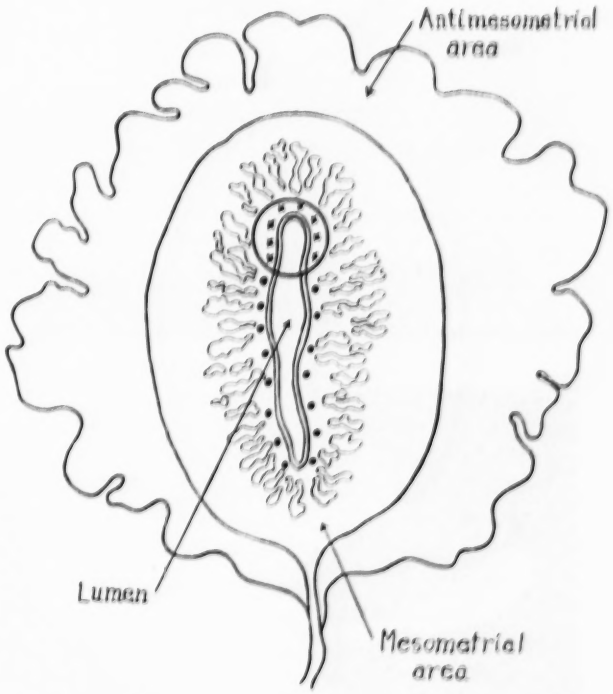
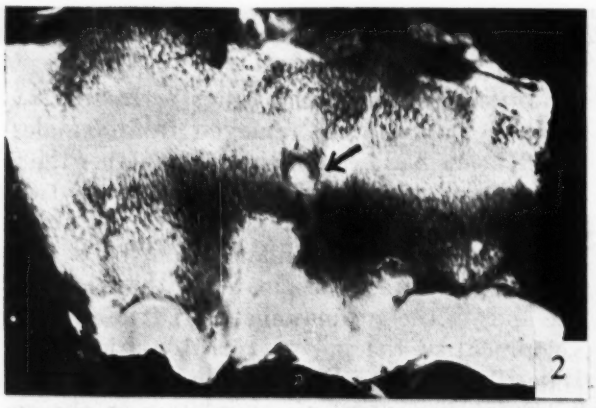
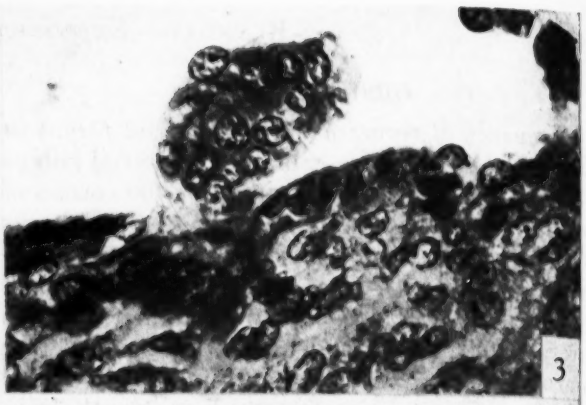
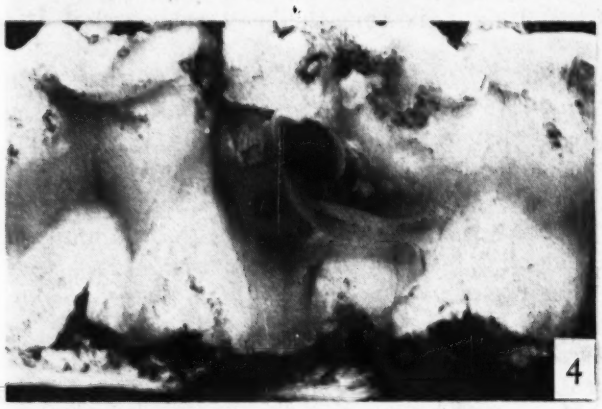


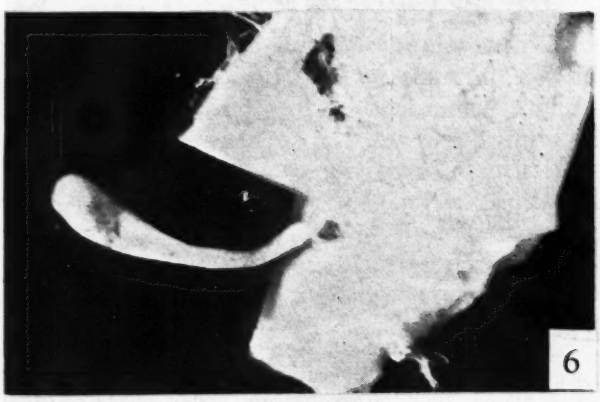
FIG. 1.—Diagrammatic representation of a cross section of a guinea pig's uterus indicating the regions where beads had been placed. All of the experimental polyps developed from the area within the central circle.

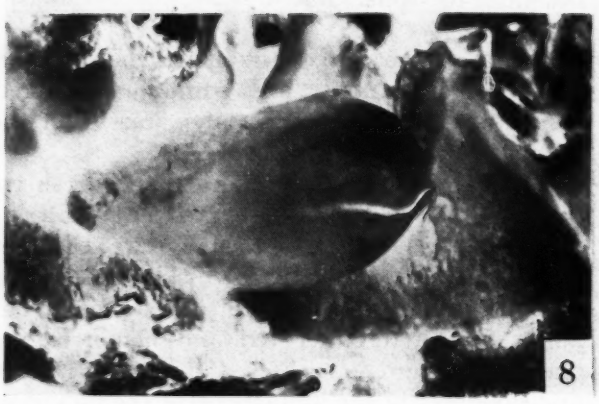
When polyps were examined in the fresh condition, a high degree of vascularity was noted. In most instances the base of the polyp was composed almost entirely of large blood vessels; the body and bulbous end, at this stage of development, ordinarily appeared either red and hemorrhagic or black and gangrenous (Figs. 4, 6, and 7). Necrosis and sloughing of the bulbous tip of the polyps was responsible for intrauterine hemorrhages in approximately 30 per cent of the cornua containing these growths.











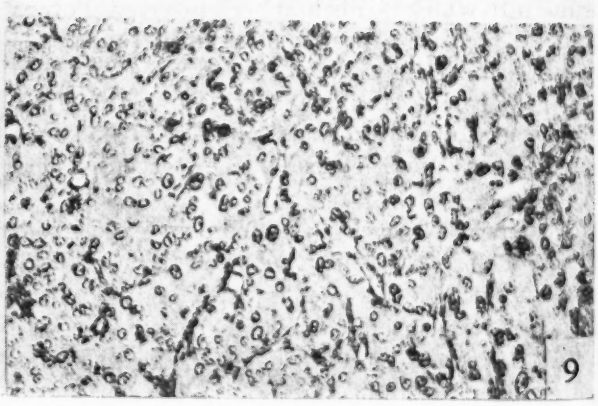


FIG. 2.—Whole mount of a portion of a fresh uterus slit open along the mesometrial border. This specimen was removed from a normal guinea pig killed on the 6th day of the reproductive cycle. Arrow points out the polyp situated within the apex of the antimesometrium. ×7.

FIG. 3.—Longitudinal section through an endometrial polyp removed from a normal guinea pig on the 5th day of the cycle. Hematoxylin and eosin. ×450.

FIGS. 4 THROUGH 8.—Typical examples of experimentally induced endometrial polyps. In each case the uterus was slit open along the mesometrial border. The cornua in Figures 4 and 6 had been fixed in Bouin's fluid; those in Figures 5, 7, and 8 were photographed while fresh. ×7.

FIG. 9.—Histologic section through an endometrial polyp which had been removed from one of the experimental animals. Note the dilated capillaries. Hematoxylin and eosin. ×450.

The histologic characteristics of the polyps varied somewhat depending upon the extent of involvement of the endometrium during the growth of the polyp and on the amount of hemorrhage into the stroma. Every polyp in the experimental group was completely covered by a layer of epithelium. Near the base of each polyp this epithelium was ordinarily cuboidal and stratified but as the polyp increased in length the superficial epithelium became much flattened. In the actively growing polyps the stroma consisted of closely packed cells with large vesicular nuclei. The cytoplasm was dense and stained quite basophilic when compared to areas of the endometrium not immediately involved (Figs. 9 and 10). During the early stages of development the stroma contained a rich capillary bed which was almost sinusoidal in nature (Fig. 9). Mitotic figures were infrequent except within the endothelium of the blood vessels. The histologic picture of the polyps in general simulated closely that seen in a decidual response initiated by an implanting ovum. As already mentioned hemorrhages were frequently seen within the stroma especially in the more distal portion. These hemorrhages were associated with a rapid necrosis and sloughing of the tissue and in certain instances were the site of uterine bleeding.

The very localized origin of the polyps may be seen in Figures 10 and 12. The base of the rather large polyp shown in Figure 12 was composed almost entirely of blood vessels. It is of interest that the stroma underlying the base of most of the polyps retained, more or less, its normal histologic characteristics (Figs. 10 and 12). In Figure 12 there is considerable hemorrhage in the entire peripheral areas and when examined in the fresh state the entire polyp appeared gangrenous. When sectioned the central area, although free from hemorrhage, nevertheless showed that the majority of cells were undergoing cytolysis.

If more than the most superficial stroma of the endometrium was involved in polyp formation the deeper glands were incorporated within its base. They hypertrophied and became cystic (Figs. 11 and 15). At times, in addition to the dilated glands within the base of the polyp, other cystic glands were found immediately surrounding the point of origin.

The location of the bead in relation to the polyp varied considerably. In the majority of instances it was located within the stroma distal to the base (Figs. 10 and 12). If, on the other hand, the bead originally lodged in the deeper layers of the endometrium it remained at the site of implantation and was not incorporated in the polyp as it developed.

The polyps ordinarily underwent cytolysis rapidly after the twelfth day of the cycle and had completely lost contact with the endometrium by the time of the onset of the succeeding heat period. The polyps were not resorbed but became detached at the base and came to lie free within the uterine lumen. In several instances they had partially passed through the cervix and in one case the elongated, whitish remnant was pulled from the vagina during the succeeding heat period (Fig. 14). Histologically the degenerating polyps appeared as meshworks of connective tissue fibers enclosing the remains of the cytolyzing cells (Fig. 13).

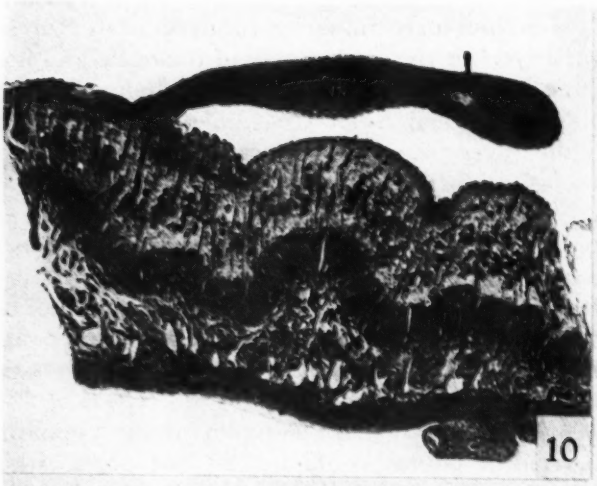
It is of interest that by employing the technique described, polyps could be induced only within the area of a few millimeters of the apex of the antimesometrium (Fig. 1), and only in response to beads implanted between the second and seventh days of the cycle. Figure 16 is a section of a cornu in which two beads had been implanted in the area of the apex of the antimesometrium on the tenth day and the animal killed for examination on the fifteenth day. There was no evidence of polyp formation, nor was there any visible disturbance of the endometrium as the result of the needle track. It is an interesting correlation that the antimesometrial area is the usual site of implantation in the guinea pig, also, as indicated by these experiments, this is the only area that can be stimulated to produce polyps.

DISCUSSION

There is no absolute etiological factor which has been shown to be the cause of endometrial polyp formation. It has been suggested that the immediate cause of the hyperplasia may be due to an excessive stimulation of the endometrium with estrogen. This is probably the best explanation of the cases of polypoid hypertrophy as described in the human (6, 7). Others have indicated that localized inflammatory changes may cause endometrial hyperplasia in a circumscribed area which eventually results in polyp formation (2). It seems likely that localized stimulation of the endometrium during the corpus luteum phase of the cycle in the human would also result in polypoid growths similar to those described in the guinea pig. It can only be conjectured whether after they had formed they would persist or slough off at the end of the cycle.

With the exception of the human, endometrial polyps occur only rarely in mammals. In the latter they ordinarily do not reach a significant size, nor have they been demonstrated to be the cause of persistent hemorrhage.

In consideration of the time in the reproductive cycle that endometrial polyps can be induced in



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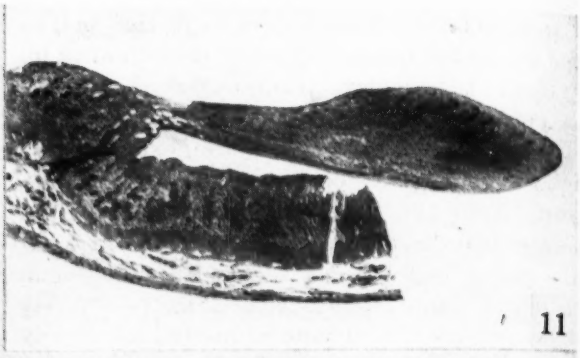

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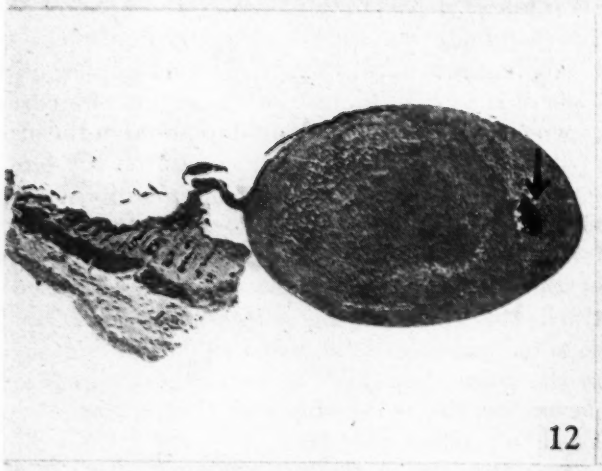

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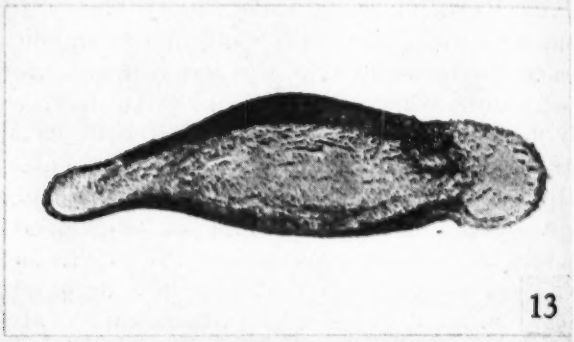

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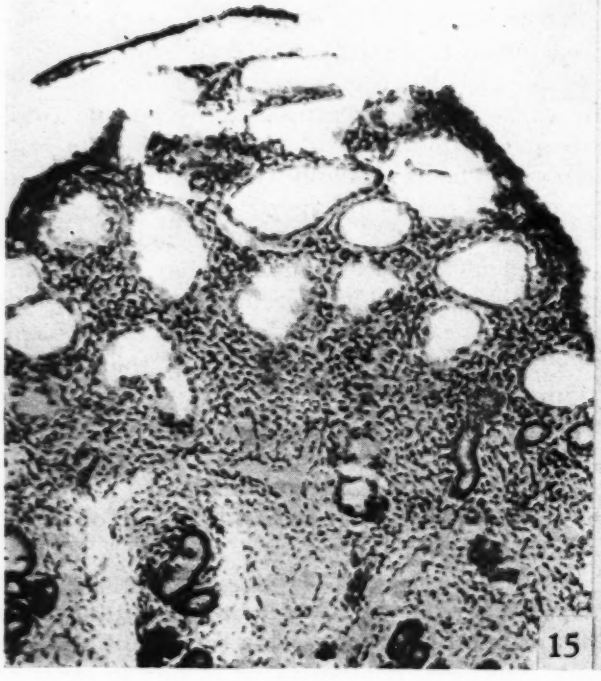

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FIGS. 10–16

the guinea pig; their histologic characteristics, and the length of time during which they persist, it is believed that these polyps represent abortive decidual reactions in a localized area. They have several characteristics however, which are ordinarily not seen in a deciduoma induced by the usual experimental means (8). In the first place their localized origin with the minimal disturbance of the underlying stroma emphasizes the potentials of growth of the more superficial layers of the endometrium. Secondly, when the deeper layers became involved and the glands of the stratum basalis were included in the growth, they hypertrophied and became cystic. It is interesting that the passage of the needle without deposition of a bead failed to induce polyps in any region of the endometrium, irrespective of the time in the reproductive cycle that it was inserted. On the other hand, the presence of a small bead, deposited with the same needle and located in the apex of the antimesometrium, invariably induced a polyp, if inserted in the proper time of the cycle. Thus if a polyp is to be induced at all there must be, either a greater initial stimulus, or the stimulus must exert its influence over a longer span of time. It is known that the stimulus necessary to induce a decidual reaction varies from species to species and need not be specific (9, 10). Of particular interest is the regional variation in the sensitivity of the endometrium to the specific stimuli used here. To date there is no specific histologic or cytologic information which would give a clue as to the meaning of this regional difference.

Examination of the sites of normal implantation (11, 12) and the experimental reversal of the mesometrial-antimesometrial axis in the rat (13) indicate that in the majority of animals at least, there is a definite polarity with respect to the ovum-endometrial interrelationship. It is important to investigate the role of each in the determination of this polarity.

SUMMARY

1. Endometrial polyps occur rarely in the normal guinea pig and do not grow to a large size.

2. Polyps may be experimentally induced by inserting small inert objects into the immediate subepithelial stroma of the endometrium in the antimesometrial area between the second and seventh days of the reproductive cycle.

3. The polyps thus produced resemble localized deciduomata to some extent. They differ from other deciduomata in that hemorrhages frequently occur into the stroma of the polyps. Necrosis and sloughing of the tips of the polyps resulted in uterine bleeding in 30 per cent of the polyps examined.

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FIGS. 10 THROUGH 12.—Longitudinal sections through uteri containing experimentally induced endometrial polyps. Note the localized origin of the polyps and the minimal alteration of the subadjacent endometrium. Arrows point out the location of the beads in Figures 10 and 12. Hematoxylin and eosin. ×17.

FIG. 13.—Section through a polyp which had lost contact with the endometrium. It consists primarily of delicate connective tissue within which still may be seen the remains of some of the degenerating cells. Hematoxylin and eosin. ×17.

FIG. 14.—Appearance of fresh specimen of polyp which had lost attachment with the endometrium. It was removed from the vagina of an experimental animal at the onset of the succeeding heat period. ×13.

FIG. 15.—Hypertrophy and cystic nature of the glands at the base of an experimentally induced polyp. Hematoxylin and eosin. ×450.

FIG. 16.—Cross-section through a cornu of an experimental animal in which two beads had been placed subepithelially in the antimesometrial area on the 10th day of the cycle and the animal killed at the onset of the succeeding heat period. There is no indication of reaction of the endometrium or polyp formation. Hematoxylin and eosin. ×450.

Mechanism of Induction of Ovarian Tumors by X-Rays*

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Tumors have developed in ovaries grafted into the spleen or pancreas of gonadectomized mice or rats (1, 8, 11 to 14). Such tumors did not appear when animals bearing such grafts possessed a functional gonad in the normal position, or if adhesions formed between the spleen and body wall so that venous drainage was into the systemic circulation, or if estrogenic hormone was administered (14). Estrogenic hormone produced by the ovary in the splenic or pancreatic position drains directly into the portal venous system, and in these species is to a great extent inactivated by the liver before reaching the systemic circulation (9).

The gonadotrophic content of the pituitary gland of gonadectomized rodents is elevated (3, 4).

TABLE 1

Data on Development of Ovarian Tumors in Bagg Albino Mice Following Radiation with 200 r of X-Rays

No. of mice	Method of radiation with 200 r	No. with tumors in radiated ovaries
4	Local to each ovary	4
2	Local to one ovary—2d ovary extirpated	2
15	Local to one ovary—2d ovary untreated	0
24	Whole body	24

Mice possessing ovarian tissue whose secretion passes directly into the portal venous system are physiologically castrate. Consequently it has been postulated that the stimulus responsible for the genesis of tumors in grafted ovaries is gonadotrophic hormone, which may be secreted in increased quantity by the pituitary gland if this organ is not inhibited by gonadal secretion.

Ovarian tumors may be induced in mice by whole body exposure to x-rays. These tumors are of the same histological types as those which develop in ovaries grafted into the spleen, that is, tubular adenomas, granulosa cell tumors, and luteomas (6, 7). Radiation with x-rays may reduce estrogenic homone output of the ovaries, thereby affecting the gonadotrophic output of the pituitary gland. Thus, the mechanism of induction of tumors might then be the same as in the splenic grafts, the gonadotrophic hormone acting upon the remaining ovarian tissue of reduced estrogenic secretory activity.

The purpose of these experiments was to determine whether x-rays *per se* induce ovarian tumors in mice in the presence of normal ovarian function.

MATERIALS AND METHODS

The Bagg albino stock of mice is very susceptible to the induction of ovarian tumors by x-rays. Practically all females receiving 200 r by whole body radiation at 8 to 10 weeks of age possess bilateral ovarian tumors if they survive 16 months post-irradiation (10). Females of this stock were placed on experiment in the following groups (Table 1):

I. Each ovary of 4 Bagg albino females received 200 r by contact radiation.
II. One ovary was radiated with 200 r, the other extirpated in 2 mice.
III. One ovary was radiated, the other untreated in 15 mice.

All animals were 8 to 10 weeks of age at the time of radiation. Originally the groups of mice were larger, but those developing pneumonia were sacrificed early and excluded from consideration.

In radiating the animals the entire mouse was shielded by lead except for a tiny aperture through which the ovary to be radiated was drawn. The ovary was placed in position on the lead plate covering the mouse at the time of radiation. When both ovaries were radiated the operation was done in 2 stages with a week's interval between exposures. The ovaries were radiated with a Phillips Contact Therapy Machine (45 KV, 2 ma., 1 mm., aluminum filter, 19 mm. target skin distance, 1500 r per minute). The period of exposure at 1500 r per minute was 8 seconds.

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† Submitted in partial fulfillment of the requirements for the degree of Master of Science at the University of Minnesota.

All the mice except one were maintained as virgins, and vaginal smears were examined 12 and 16 months after radiation (Table 2). Between 16 and 17 months after radiation the animals were autopsied. Sections of the ovaries, adrenals, reproductive tracts, and submaxillary glands were studied.

OBSERVATIONS

Ovarian tumors were present in all of 24 Bagg albino females within 16 months following whole body radiation of mice 8 to 10 weeks of age with 200 r of x-rays. Larger doses of x-radiation produced similar results. Microscopically these tumors varied in structure, but were usually composed of surface epithelial downgrowths and proliferations of granulosa cells among which were nests of luteal cells (Fig. 2). Varying portions of the ovary contained cells filled with ceroid pigment (Fig. 1). This pigment may be deposited within either tumor cells or normal ovarian luteal cells.

Grossly, ovaries containing tumors were larger than normal, varying from approximately 3 mm. to over one cm. in diameter, and were usually yellow in color. In some instances the ovary contained cysts in addition to the yellow tumor masses. Cysts were filled with either clear fluid or blood. An animal died occasionally from hemorrhage into the peritoneal cavity.

When both ovaries were radiated individually with 200 r of x-rays by contact radiation, no other portion of the body being exposed to radiation, bilateral tumors appeared (Table 1). If one ovary was radiated in the same manner, and the other ovary removed, tumor development resulted (Table 1). In all of 15 cases where a single ovary was radiated with 200 r and the other ovary untreated, no tumor was induced in the radiated ovary (Table 1). Such ovaries presented a characteristic appearance 16 months post-radiation (Figs. 3 and 4). They were smaller than the non-irradiated ovaries. Microscopically they were composed of a cortex of anovular follicles (Figs. 3 and 4), and a medulla containing stromal cells, some of which contained ceroid pigment. Surface epithelial downgrowths were not present. The follicles appeared to be those which had persisted following destruction of the ovum by radiation. There was no evidence of new formation of anovular follicles from the surface epithelium.

One of the non-irradiated ovaries (in animals in which the other ovary was radiated) contained a granulosa cell tumor (Figs. 7 and 8). Of particular interest is the fact that although this ovary was almost completely replaced by tumor within it, at least two fully developed ovarian follicles were present. A second non-irradiated ovary contained large hemorrhagic cysts, and in a third epithelial downgrowths were present, and granulosa cell proliferation was evident immediately beneath the surface epithelium (Figs. 9 and 10). The remaining non-irradiated ovaries contained a few well developed ovarian follicles, with a considerable portion of the ovary being composed of stromal cells, many containing ceroid pigment (Fig. 5). No anovular follicles were present in the cortex (Fig. 6).

The incidence of spontaneous ovarian tumors in the Bagg albino stock is very low. No ovarian tumors were seen in any of 17 females beyond 17 months of age.

Data on vaginal smears of test animals are given in Table 2. Twelve months post-irradiation the vaginal smears of animals whose ovaries had been radiated by contact radiation were castrate, whereas 12 of 15 of those in which only a single ovary had been radiated were undergoing cycles. Sixteen months after radiation cycles were still evident in 7 of 15. At this age cycles are disappearing in intact, untreated Bagg albino females.

TABLE 2

DATA ON VAGINAL SMEARS OF BAGG ALBINO MICE RECEIVING 200 r OF X-RADIATION

No. of mice	Method of radiation with 200 r	Age post-irradiation	Number cycling	Number castrate
4	Local to each ovary (one week apart)	12 months	0	4
2	Local to one ovary—other extirpated	" "	0	2
15	Local to one ovary—other untreated	" "	12	3
4	Local to each ovary (one week apart)	16 months	0	4
2	Local to one ovary—other extirpated	" "	0	2
15	Local to one ovary—other untreated	" "	7	8

DISCUSSION

X-radiation of a single ovary did not induce ovarian tumors if the second ovary was not radiated. This strongly suggests that the mechanism of induction of ovarian tumors is similar to that operating in the development of tumors within ovaries grafted into the spleen, where the presence of a functioning ovary in the normal position inhibits tumor formation in the grafted ovary. When all of the ovarian tissue was radiated, the estrogenic secretion of the ovary was largely abolished within at least a few months post-irradiation, although some Bagg albino mice bear litters following radiation with 200 r. Estrous cycles have been reported in mice made anovular by roentgen irradiation (2). Furthermore, ovarian tumors have been induced in mice receiving only 87 r of x-rays (5). Complete withdrawal of ovarian estrogenic

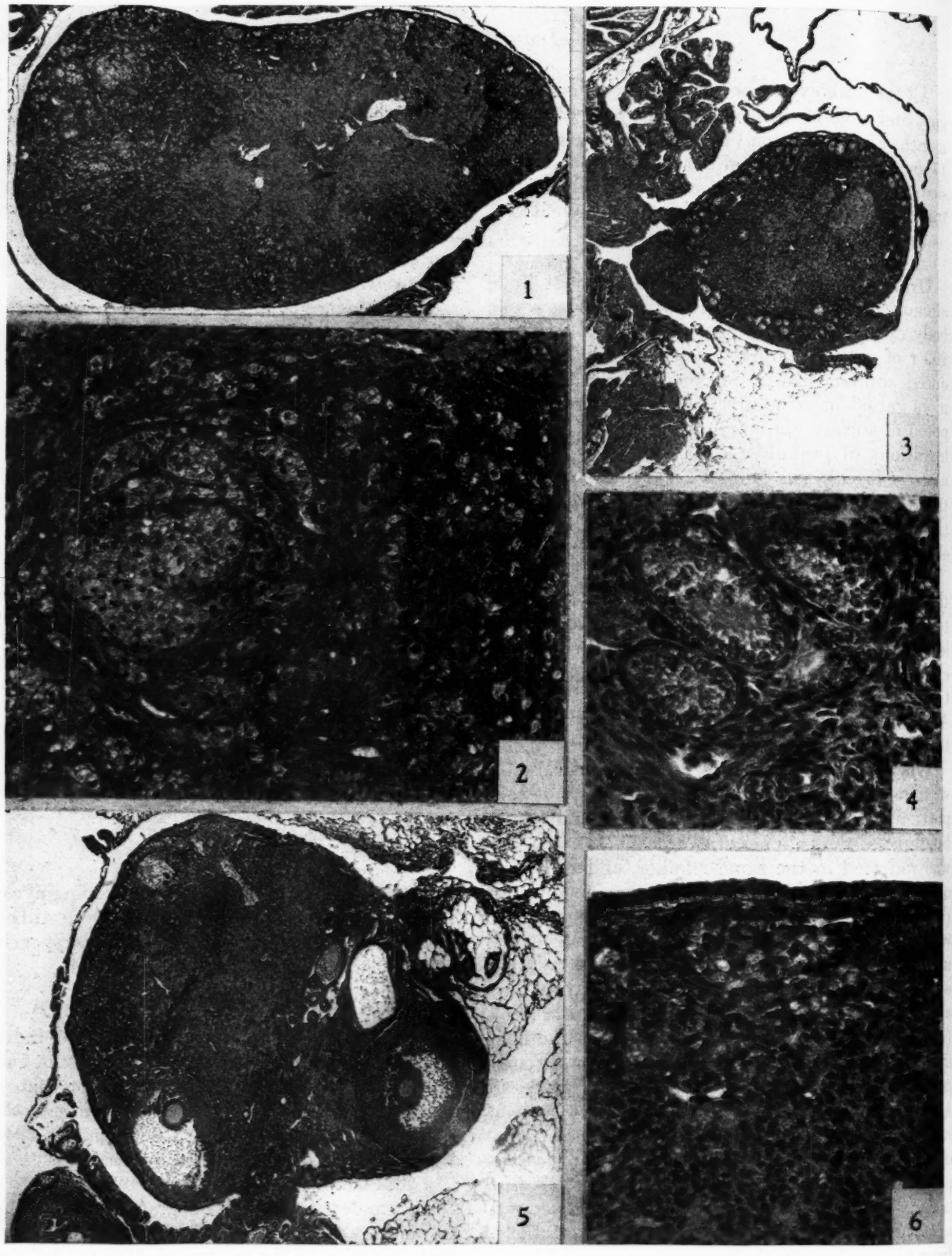

FIG. 1.—Ovary of Bagg albino female; both ovaries radiated with 200 r of x-rays by contact radiation 16 months prior to autopsy. Most of cortex invaded by granulosa cell tumor shown in Figure 2. Central portion contains cells with ceroid pigment. Ovary grossly enlarged. Mag. ×40.

FIG. 2.—High power view of tumorous portion of ovary illustrated in Figure 1. Dark cells are granulosa cells; those with more abundant pale cytoplasm are luteal cells. Mag. ×70.

FIG. 3.—Ovary of Bagg albino female radiated with 200 r by contact radiation 16 months before autopsy. Other ovary not radiated, and shown in Figure 5. Cortex composed of anovular follicles. Central portion has fibroblastic stroma, some of the cells containing ceroid pigment. Tumors did not develop in radiated ovaries if the second ovary was not radiated and remained functional. Grossly this ovary was smaller than the non-radiated ovary. Mag. ×40.

FIG. 4.—Anovular follicles from the cortex of the ovary shown in Figure 3. Mag. ×150.

FIG. 5.—Non-irradiated ovary from animal whose other ovary, shown in Figure 3, was radiated with 200 r 16 months before autopsy. Mature follicles still present. Mag. ×40.

FIG. 6.—Cortex of non-irradiated ovary shown in Figure 5. No anovular follicles present as in radiated ovary illustrated in Figure 3. Mag. ×150.

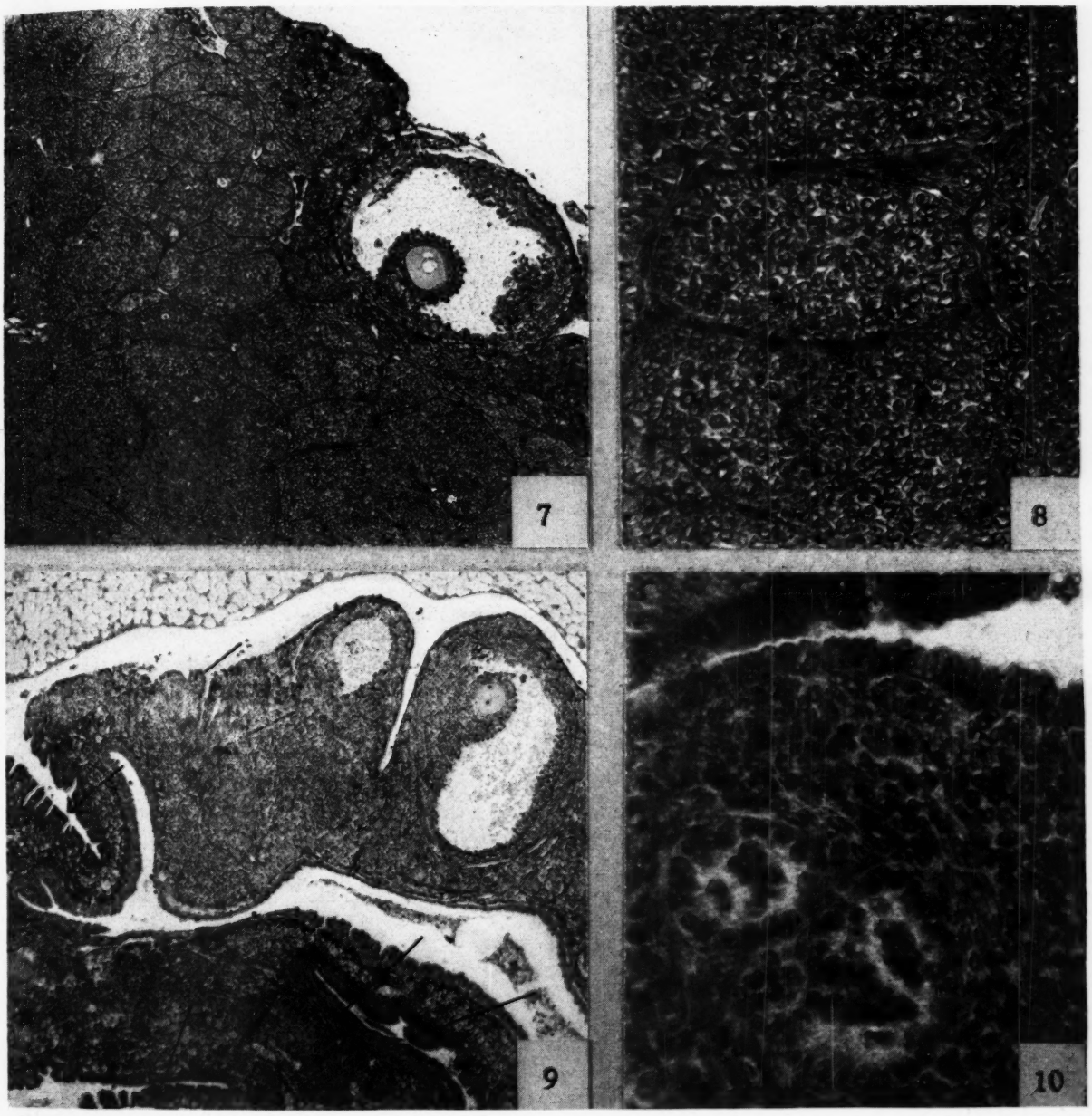

FIG. 7.—Granulosa cell tumor in non-irradiated ovary of a Bagg albino female whose other ovary was radiated with 200 r of x-rays by contact radiation 16 months before autopsy. Except for two mature follicles enlarged ovary is composed of cords of granulosa cells. Mag. ×60.

FIG. 8.—High power view of granulosa cell cords shown in Figure 7. Mag. ×180.

FIG. 9.—Non-irradiated ovary of animal whose other ovary received 200 r of x-rays 16 months before autopsy. Arrows indicate areas of granulosa cell proliferation and surface epithelial downgrowth. Mature follicles are present. Mag. ×60.

FIG. 10.—High power view of area of granulosa cell proliferation in ovary illustrated in Figure 9. Mag. ×180.

secretion by radiation is thus not essential for the induction of ovarian tumors.

The development of ovarian tumors or tumor-like proliferations in the second non-irradiated ovary, 3 of 15, may be significant. To determine whether reduction in estrogenic secretion resulting from radiation of a single ovary plays a role in the genesis of such tumors, the effect of extirpation of a single ovary on tumor formation in the remaining ovary is being tested.

SUMMARY

X-rays did not induce tumor development in a radiated ovary of the Bagg albino stock if the animal's second ovary remained unirradiated and functional. Contact radiation of a single ovary induced ovarian tumor development only if the second ovary was extirpated. Bilateral contact radiation resulted in tumor development. Although a second functioning ovary inhibited ovarian tumor development following unilateral contact radiation, a granulosa cell tumor developed spontaneously in a non-irradiated ovary containing two fully developed ovarian follicles.

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Studies on Lymphocystis Tumor Cells of Fish

I. The Osmiophilic Granules of the Cytoplasmic Inclusions and their Interpretation as Elementary Bodies of the Lymphocystis Virus

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The lymphocystis disease of fish is outstanding among other virus diseases because of the enormous growth of the infected host cells and the long period of their life. In the European flounder *Pleuronectes flesus*, for instance, infected fibroblasts transformed into round cells, the so-called lymphocystis cells, reach a diameter up to 2000 microns during a period of one year. Corresponding to the gigantic size attained by the host cells, cytoplasmic inclusion bodies reach a much higher level of development in size, shape, and differentiation than in other virus diseases. Thus the lymphocystis material offers the opportunity to study the cellular pathology of a virus disease under unusually favorable conditions of time and space.

Although lymphocystis inclusion bodies in some fish families assume the peculiar shape of a network of enormous size, a number of facts in their development indicates that they indeed correspond to cytoplasmic inclusions in other virus diseases. They grow from tiny initial corpuscles, as is known, for instance, in the development of the Guarnieri bodies in vaccinia or the Negri bodies in rabies. They pass through a stage in which they resemble very much the compact stage of the Guarnieri bodies of vaccinia. Then they become vacuolated and assume an aspect which reminds one of the vacuolated plaques observed in lymphogranuloma venereum by Rake and Jones (7) or described in certain plant virus diseases, *e.g.* by Kunkel (6, p. 364, Fig. 10) in the cells of the sugar cane in the Fiji disease.

It has to be admitted, however, that characteristic features which in the last twenty years have gained more and more interest and importance in the morphology of viruses have not yet been described in lymphocystis. These are the elementary bodies, for which ample evidence has been obtained in the studies on vaccinia, fowl-pox, psittacosis, lymphogranuloma venereum, and other viruses to show that they represent virus units which serve as the transmission stage of the virus.

The difficulty in finding and discerning elementary bodies in the lymphocystis cells was not due to a lack of visible granules in these cells, but consisted in the difficulty of differentiating specific granules from ordinary granular structures in the cytoplasm. As I resumed the study of the finer structures in the lymphocystis inclusion bodies in the last years, I have, however, succeeded in demonstrating special granules which take their origin in the inclusion bodies and increase enormously in number in the growing lymphocystis cells. The reasons for the interpretation of these granules as the transmission stages of the virus will be discussed in the present paper. The observation of an intensive staining reaction of the granules with osmic acid has opened the way to identify them and to study their development.

Several methods, in the course of which the tissue is kept in an osmic acid solution for several days, gave essentially similar results. The first technique used was the method developed by Kopsch (5) for the study of the Golgi apparatus. Small pieces of fresh material are put into a 2 per cent aqueous solution of osmic acid for 7 days and kept at a temperature of 37° C. Then they are washed in distilled water, and after dehydration in alcohol and passage through xylol, embedded in paraffin for sectioning. In this procedure the osmic acid not only stains the tissue but first acts as a fixative. Modifications in which the material is fixed otherwise and secondarily exposed to osmic acid for staining also gave positive results. For instance, I used the method of Sjövall (9) in which the tissue is fixed in a mixture of formalin (1 part) and distilled water (3 parts), and after washing in distilled water is placed in the osmic acid solution

for 3 days at 37° C. Further I found that such secondary application of osmic acid gives good staining results also after fixation of the tissue with the ordinary 10 per cent formalin solution, even when the material had been kept for years in formalin ("formalin-osmium method"). Also in tissue fixed in acetified alcohol, Schuberg (8), or in Flemming's fluid, positive staining results were obtained by placing the material in osmic acid solution for several days.

In preparations in which a positive osmic acid reaction has occurred the various constituents of tissue and cells are stained in characteristic shades varying from a pale yellowish color to dark brown or black. In lymphocystis cells in which the osmium reaction has turned out typically the specific granules are outstanding as dark brown structures. By their intensive reaction with osmic acid they contrast distinctly with a less intensively stained framework of the inclusion bodies as well as with the palely stained cytoplasm. Relatively rarely the cytoplasm of lymphocystis cells contains coarse granular cell products which likewise react intensively with osmic acid. Usually they can be distinguished from the dark brown granules as deeply blackened structures. Mitochondria which might represent a possible source of error seem to be present only in young stages of lymphocystis cells. This complication can be avoided by using acid fixatives. As another complication it may be mentioned that sometimes, especially in deeper layers of a tumor piece, the whole cytoplasm of the lymphocystis cells is stained black. It may be that such an effect indicates that the material has been exposed too long to the osmic acid.

The clear results obtained in the majority of the specimens show the osmium methods to be very useful, in spite of the fact that they take up time and are expensive. However, the intensive osmium reaction of the granules represents a specific staining reaction of virus particles no more than any of the other methods recommended hitherto for the staining of virus granules. Not only normal cell constituents such as the Golgi apparatus or mitochondria are intensively stained by the osmium but also other microorganisms, for instance bacteria, can under the same conditions give an intensive osmium reaction.

The inclusion bodies of the lymphocystis cells first become visible in the cytoplasm as tiny compact corpuscles of intensively basophilic staining reaction. During their continuous growth the basophilic inclusion substance stains less intensively and becomes interspersed with vacuoles. It depends on the type of fixation whether the substance now appears as a cake substance which encloses vacuoles (Fig. 1) or as a system of thin-walled alveoli which contains a great number of vacuoles of various sizes. In advanced stages the vacuoles have disappeared and the inclusion bodies are differentiated into two components, a framework of very intensively basophilic staining reaction and a delicate ground substance (Fig. 2). The framework consists of thin lamellae of firm consistency. In sections it can appear as a network. Often the lamellae form the walls of communicating spaces shaped like tunnels. The ground substance which fills these spaces and also covers the outer surface of the system of lamellae stains slightly with hematoxylin or safranin, but shows an acidophilic reaction when stained by the Biondi method. The ground substance appears as a deli-

Figures 1 to 5, and 7 to 9, are drawings of sections through cytoplasmic inclusion bodies from lymphocystis cells.

FIG. 1.—From a *Pleuronectes* cell of about 150 microns in diameter. Sjövall's method. ×1125.

FIG. 2.—From a *Pleuronectes* cell of 550 microns. Fixation with chromic and osmic acid (method of Meves). Staining with safranin and Cajal's mixture of indigo carmine and picric acid. ×1125. (f) framework consisting of basophilic inclusion substance; (s) outer layer of ground substance; (t) ground substance in spaces between the lamellae of the framework; arrow marks area where the framework has begun to break down.

FIG. 3.—From a *Pleuronectes* cell of 550 microns. Method of Kopsch. ×1125.

FIG. 4.—Small piece of a corresponding inclusion body. Method of Kopsch. ×1750. (f) framework; (o) osmiophilic granules.

FIG. 5.—From a *Pleuronectes* cell of 300 microns. Formalin-osmium method. ×1750. (c) cytoplasm; (i) indentations of the surface of the inclusion body; (v) vacuoles.

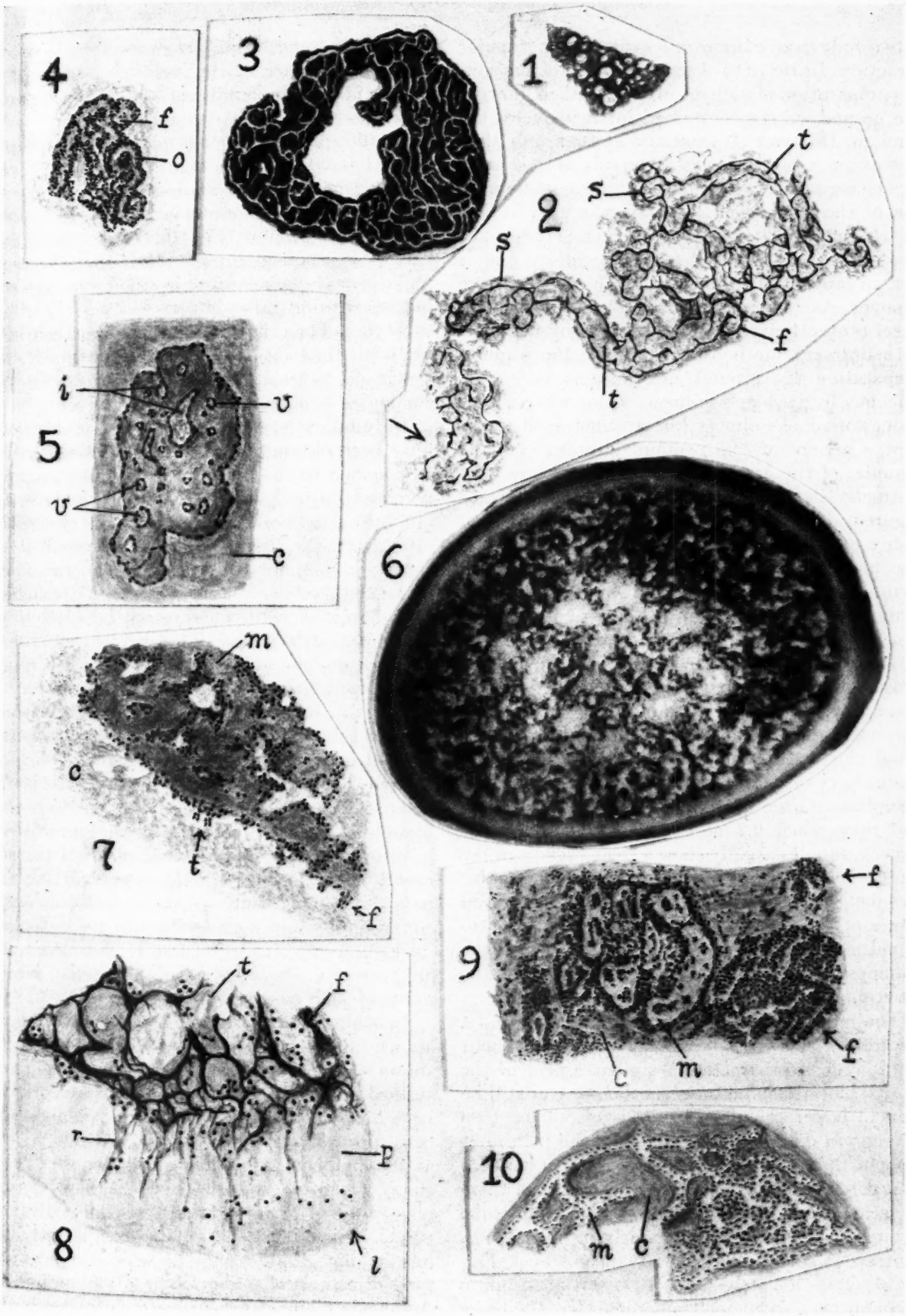
FIG. 6.—Photomicrograph of a marginal section through a *Stizostedion* cell of 205 microns in length. Sjövall's method. ×642.

FIG. 7.—Small portion of an inclusion body from a stunted *Stizostedion* cell of 125 microns in diameter. Sjövall's method. ×1750. (c) cytoplasm; (f) fringelike process of basophilic inclusion substance containing osmiophilic granules; (m) basophilic inclusion substance; (t) tetrads of granules.

FIG. 8.—Small portion of an inclusion body from a stunted *Stizostedion* cell of 150 microns. Formalin-osmium method. ×1750. (f) dense basophilic inclusion substance; (l) osmiophilic granules lying directly in the cytoplasm; (p) cytoplasm; (r) fringes of palely stained inclusion substance projecting into the cytoplasm; (t) palely stained inclusion substance.

FIG. 9.—From an *Acerina* cell of 400 microns. Method of Kopsch. ×1125. (c) cytoplasm; (f) clusters of osmiophilic granules scattered through the cytoplasm; (m) basophilic substance of an intact portion of an inclusion body.

FIG. 10.—From a squeezed preparation of a fresh *Stizostedion* cell to which dilute acetic acid was added. ×1125. (c) cytoplasm; (m) framework consisting of basophilic inclusion substance.



Figs. 1–10

cate cloudy mass of more or less distinctly granular structure. In 1921 (12) I gave a picture of an osmium preparation of such an advanced stage showing the ground substance stained intensively by the osmium. However, I presumed at that time that this osmium reaction was the result of degenerative changes. Now, in renewed studies, systematic use of the osmic acid methods has shown very clearly that the intensive osmic acid reaction of the ground substance occurs rather regularly and is not in any way connected with degenerative changes. As Figure 3 demonstrates, the staining effect is exactly the opposite of the result obtained with ordinary methods (Fig. 2). In the osmium preparation the ground substance is very conspicuous by its blackish-brown color whereas the framework looks almost like an unstained structure. Carriers of the osmium staining are the granules of the ground substance which are very distinctly shown in such osmium preparations, as Figure 4 demonstrates with higher magnification. Many of the granules have approximately equal size measuring about 0.375 microns. But larger forms have also been observed. The mass of the osmiophilic granules fills the spaces between the lamellae of the framework and covers the outer surface of the inclusion bodies as a stratified layer. Most of the granules stick to the inclusion bodies. Some, however, may be found in the cytoplasm between the inclusions, either as single granules or in small clusters. Groups of two granules or short chains have rather frequently been observed in the cytoplasm. Often such granules are larger forms and may reach 0.7 or even 1.25 microns. The largest granules usually show a clear vacuole in the center. In 1921 I interpreted such groups of granules between the inclusion bodies of advanced stages of *Pleuronectes* lymphocystis cells as mitochondria. Now I consider them as osmiophilic granules which have become detached from the covering layer of the inclusions.

The inclusion bodies shown in Figures 3 and 4 are from *Pleuronectes* lymphocystis cells of about 550 microns in diameter. A similar aspect of the finer structure of the inclusion bodies can still be seen in larger lymphocystis cells of 800 to 1500 microns in diameter. In such cells the inclusions have further grown in size or their number has continued to increase by budding. As results of these growth processes the number of the osmiophilic granules has increased enormously in the cells. In contrast to such findings in advanced stages, Figure 5 shows an inclusion body from an osmium preparation of a cell of 300 microns. Here the basophilic inclusion substance is not yet condensed to a thin framework of lamellae, but still appears as a cake substance which encloses some vacuoles. The outer surface of the inclusion body and the surface of some indentations (i) as well as the margin of the vacuoles (v) contain a single layer of osmiophilic granules, which is not yet quite complete on the left side of the surface contour. In still earlier stages only a few granules have been observed in the inclusions or not any osmiophilic granules at all have been found (Fig. 1). Thus, the study of various stages leads to the result that the osmiophilic granules appear first in small numbers within the growing mass of the basophilic inclusion substance. Then their number increases considerably while the basophilic inclusion substance shows a relative decrease in volume and finally becomes condensed to a framework of thin lamellae.

In lymphocystis cells of perches similar results have been obtained with the osmic acid methods. In contrast to the findings in *Pleuronectes*, in the perch cells usually only one inclusion body sprouts out into a network which surrounds the nucleus (10, 11, 12, 13). Figure 6 shows a marginal section through a lymphocystis cell of the American perch *Stizostedion vitreum*. This oval cell has reached a long diameter of 205 microns on the 105th day of experimental infection. The convolutions of the network are covered with small groups of osmiophilic granules which also fill some of the vacuoles within the bars of the network. Taken from a stunted cell of 125 microns Figure 7 demonstrates a small part of the inclusion network which was located only on one side of the nucleus, as is characteristic for younger stages. The thick bars of the basophilic inclusion substance are covered with a layer of osmiophilic granules. Not all of them are round. Some appear a little elongated like short rods. Some short fringes of the inclusion substance containing granules protrude into the cytoplasm, for instance at (f). At (t) two tetrads of granules are seen in a similar position. They may be remnants of such fringes.

In some of such stunted cells the inclusion substance undergoes regressive changes. Figure 8 shows such an inclusion in which only the darker stained portion of the inclusion substance seems to be of firmer consistency whereas the palely stained main portion was apparently in a semifluid state at the moment of fixation, as the long fringes (r) show, which like filiform pseudopodia of a rhizopode project far into the cytoplasm. Within the palely stained portion a number of relatively large osmiophilic granules can be seen. Some are arranged in short chains or lie in a tandem position. A delicate filament sometimes connects such granules so that the impression of an arrangement like dumb-bells results. Some of the granules are seen

within the fringes. Granules which lie free in the cytoplasm, for instance at (l), can be explained as elements detached from dissolved fringes.

In some of such stunted cells rather numerous osmiophilic granules are seen within the cytoplasm. Normally an infiltration of the cytoplasm by many granules does not occur before the framework of the inclusion bodies breaks down in advanced stages. The beginning of such a process can already be seen in Figure 2 in the left area marked with an arrow. An osmium preparation of an advanced stage of a lymphocystis cell of the European perch *Acerina cernua* shows in Figure 9 at (f) how the masses of the granules finally become scattered through the cytoplasm. The continuous increase of the number of the granules during the growth period of the inclusion bodies, as observed in *Pleuronectes*, is also very evident in the lymphocystis cells of the perches.

Any suspicion that the structures described as osmiophilic granules might be artifacts produced by osmium precipitations can be dispelled by the result of a number of observations. Often the osmium reaction fails to occur in the superficial layer of a lymphocystis tumor in contrast to the osmium effect in the deeper layer. Such a difference in the osmium staining can also be seen in a large lymphocystis cell which extends through both zones. At the transition of the two layers it can then be seen that the osmium stained granules blend into unstained granules which in the superficial layer remain visible as rather strongly refractive structures. Further the granules are, under favorable conditions of fixation, sometimes distinctly discernible when stained only with hematoxylin or safranin. Furthermore, in fresh *Stizostedion* lymphocystis cells I have observed refractive granules along the bars of the inclusion network after the cytoplasm had been made transparent by adding drops of a 1 per cent aqueous solution of acetic acid (Fig. 10). When such preparations were then exposed to osmic acid solution the granules became distinctly stained brown. The effect could clearly be seen after $1\frac{1}{2}$ hours.

There are two possibilities to be considered as to how the considerable increase in the number of the osmiophilic granules during the growth period of the inclusion bodies may come about. Either it can be supposed that they are produced in increasing numbers by differentiation in the basophilic inclusion substance which during this process becomes more and more reduced in mass, or it might be presumed that the granules are elements capable of multiplication by division. The second possibility may find support in the frequent observation of granules lying in a tandem position and sometimes appearing as dumb-bells when a delicate filament connects them. In addition to such dyads, tetrads and short chains have also been observed. However, a psychological source of error cannot completely be excluded, consisting in the tendency to construct a relation of granules which might only by chance lie more or less close together. Further the tetrads shown in Figure 7 can be interpreted as groups of granules previously arranged on fringes of the inclusion substance. If, as it seems, in stunted cells the inclusion substance can show various degrees of gel consistency, connecting filaments in dumb-bell dyads could be residual matrix structures and would not necessarily suggest telophases of the division of granules.

Thus it seems to me that for the present it must still be considered an open question how the considerable increase of the number of the osmiophilic granules comes about. In any case, however, the evidence presented strongly suggests that the osmiophilic lymphocystis granules correspond to the elementary bodies known in other macroviruses. It has been shown that the osmiophilic granules are rather strongly refractive structures which are resistant to weak acetic acid. The majority are represented by tiny granules of a rather uniform size. Also some larger forms have been observed. The granules have been demonstrated in the cytoplasmic inclusion bodies as structures which in earlier stages are present in small numbers or could not be seen at all, but later increase enormously in number and finally, after the break-down of the inclusion bodies, infiltrate the cytoplasm of the host cell. Similar facts are known about elementary bodies of other macroviruses. For instance, resistance of elementary bodies against dilute acids has been referred to by Goodpasture (4) in his studies on vaccinia, fowl-pox, and molluscum contagiosum. Elementary bodies have been demonstrated as components of cytoplasmic inclusion bodies in vaccinia by Goodpasture (4) and by Bland and Robinow (3), in fowl-pox by Goodpasture (4), in lymphogranuloma by Rake and Jones (7), in psittacosis by Bedson and Bland (1, 2) and Yamamura and Meyer (14), among other authors. The observations in lymphocystis correspond very well to those of Bland and Robinow (3) in vaccinia, in so far as in both the elementary bodies could not be shown in the early stages of the inclusion bodies but were demonstrated as components of the inclusions when these bodies expanded into large networks.

It seems that heretofore no observations have been made as to how elementary bodies of other macroviruses react to prolonged treatment with osmic acid solution. But it might be worth while to

try the osmic acid methods in the study of other viruses. With an average size of 0.375 microns in paraffin sections, the small type of the osmiophilic granules of lymphocystis seems to be rather similar to the elementary bodies of lymphogranuloma for which Rake and Jones have stated a size of 0.4 microns in fixed and stained smear peparations (7).

The study of the osmiophilic lymphocystis granules has hitherto been performed only with the light microscope. The fact that the inclusion bodies of the lymphocystis cells pass through a stage in which they very much resemble Guarnieri bodies seems to indicate that the lymphocystis virus belongs to the pox group, which under the electron microscope is characterized by a brick-like shape of the elementary bodies. It will be of great interest to find out with the electron microscope if the granules of the lymphocystis virus have indeed a corresponding shape.

The basophilic inclusion substance has previously been interpreted by me (10, 12) as a reaction product of the host cell. However, as will be discussed in another paper, I have now good reasons to consider the basophilic inclusion substance as an early phase of the virus. It seems to me that the osmiophilic granules are the transmission stage whereas the basophilic inclusion substance represents the vegetative phase of the virus, which as the substance of the young inclusion bodies expands rapidly in the host cell. Later on the vegetative phase is transformed into a system of membranelles that supports and envelops masses of transmission granules which have meanwhile developed.

SUMMARY AND CONCLUSION

The lymphocystis virus disease of fish is characterized by the enormous growth of the infected fibroblasts which are transformed into the so-called lymphocystis cells. In the cytoplasmic inclusion bodies of the lymphocystis cells of flounders and perches, granules have been demonstrated which give an intensive osmium reaction when exposed to osmic acid solution for several days. The number of these osmiophilic granules increases considerably during the growth period of the inclusion bodies. It remains an open question whether this increase in number is the result of divisions of the granules or the result of a progressive differentiation of the granules in the basophilic inclusion substance. After the break-down of the basophilic framework of the inclusions at advanced stages, clusters of the granules become scattered through the cytoplasm of the host cell. Most of the osmiophilic granules are small corpuscles of a rather uniform size of about 0.375 microns in paraffin sections. In addition to this small type some larger forms have been observed. The granules studied in fresh preparations are rather strongly refractive. They are resistant to a weak acetic acid solution.

As far as can be judged on the basis of a solely morphological study with the light microscope, the osmiophilic granules of the lymphocystis inclusion bodies apparently correspond very well to the elementary bodies of other macroviruses. They are interpreted as the transmission stages of the lymphocystis virus.

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American Association for Cancer Research, Inc.

39th Annual Meeting

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SOME ENIGMAS ASSOCIATED WITH THE GENESIS OF MAMMARY CANCER IN MICE.

JOHN J. BITTNER. (Division of Cancer Biology, Department of Physiology, University of Minnesota Medical School, Minneapolis 14, Minn.)

Many of the advances in cancer research have been made possible because of controlled biological material, especially inbred stocks of mice. The results of various studies will be presented to show that, as expected, these strains are subject to mutational changes which may give various sublines with different incidences of mammary cancer. Controls should be maintained for each experimental series.

A review of the literature will demonstrate how no single theory will account for the apparently contradictory data which are being consistently observed in the same and different laboratories by the use of inbred strains on the interaction of the causative factors for mammary cancer. Additional data will be given regarding the inherited hormonal influence, the effects of force breeding, and the biological characteristics of the mammary tumor milk agent.

More effort and assistance should be devoted to the development of new techniques and methods, the analysis of various physiological phenomena, etc., which may assist in the interpretation of the experimental data.

ULTRAVIOLET CYTOCHEMICAL STUDY OF RAT LIVER CELLS UNDER DIFFERENT CONDITIONS OF NORMAL AND NEOPLASTIC GROWTH.

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The results of morphologic cellular measurements were correlated with ultraviolet cytochemical observations using the methods developed by Caspersson in Sweden for a study of rat liver cells under conditions of (a) regeneration, (b) low and high protein diets, and (c) hepatoma formation by p-dimethylaminoazobenzene. Little change in the nucleic acids of the nucleolus or cytoplasm occurs in the first 24 hours of regeneration following removal of two-thirds of the liver, although the cytoplasmic, nuclear, and nucleolar volumes increased 2.4, 2.2, and 4.1 times respectively. Following the increased mitotic activity of the second day the volume of the cellular constituents decrease toward normal and the cytoplasmic nucleic acid increases.

After prolonged dietary protein depletion, the volume of the cytoplasm decreases 22 per cent and the nucleus and nucleolus increase 1.2 and 2.8 times. The respective ratios return to normal after a high protein diet. The methods employed did not detect significant differences in the ratios of protein to nucleic acid during protein depletion or repletion.

Hypertrophic liver cells after feeding p-dimethylaminoazobenzene resemble regenerating liver cells. The hepatomas measured had cells with less cytoplasmic, nuclear, and nucleolar volumes and increased nucleic acid in both the nucleolus and cytoplasm. Some of the possible relationships of nucleic acids and nucleoli under the different conditions are discussed.

EFFECT OF SPLENECTOMY ON THE DEVELOPMENT OF MAMMARY TUMORS IN MICE CONTAINING THE MILK AGENT.

B. E. BENNISON. (Introduced by H. B. Andervont.) (National Cancer Institute, Bethesda 14, Md.)

Mammary tumors in certain strains of mice have been considered the result of the presence of a virus referred to as the milk agent. The eight to ten month latent period has stimulated attempts to experimentally alter the host-virus relationship so that tumors would develop at an earlier age.

The spleen has been shown to play an important part in immunity, and the question arose as to whether or not splenectomy, by altering the immune status of the host, would affect the rate of development of milk agent induced mammary tumors.

Strain C female mice, whose mothers contained the milk agent, were divided into experimental and control groups. Experimental animals were splenectomized under ether anesthesia at about one month of age. Litter-mate controls were subjected to laparotomy in which the spleen was externalized and returned to the abdomen. All mice were kept under similar conditions and observed for the development of mammary tumors.

As compared with the controls, the splenectomized mice showed a longer latent period and a lower total incidence of mammary tumors. The observations are discussed with reference to the relation of the spleen to infection with the milk agent.

MAMMARY GLAND TUMORS IN A LINE OF C3H MICE DEPRIVED OF THE MILK AGENT. WALTER E. HESTON, MARGARET K. DERINGER (by invitation), and WAYNE D. LEVILLAIN (by invitation). (National Cancer Institute, Bethesda 14, Md.)

This line referred to as C3Hb was started from a litter of 3 males and 3 females taken by cesarean section from their high-tumor strain C3H mother with the milk agent, and foster-nursed by a low-tumor strain C57 black female without the agent. Subsequently the line has been maintained by brother × sister matings the young being nursed by their own mothers. A total of 218 females of the line extending through 5 generations have reached 12 months of age or more. To date 20 or 9 per cent of these females have developed mammary tumors and these appeared at an average age of 15.5 months; 49 have died without tumors at an average age of 14 months; and 149 are living without tumors at an average age of 15.7 months. The histologic sections of some of these tumors showed squamous cell areas with pearl formation representing a type rarely found in C3H females with the agent, although others were not unlike the usual types of C3H mammary gland tumors. Thus far there is no evidence that these tumor-bearing females had the milk agent comparable to the original strain C3H, either from the distribution of the tumor-bearing females on the pedigree chart or from tests of cell free extracts of the tumors injected into young C3Hb females. Final conclusion, however, cannot be drawn at this time since the test females are still living although those for three of the tumors are beyond the expected tumor age.

PRELIMINARY STUDY OF THE CYTOTOXIC ACTION OF THE MAMMARY TUMOR AGENT ANTISERUM ON MAMMARY CANCER CELLS. L. W. LAW. (National Cancer Institute, Bethesda 14, Md.)*

It has been reported by Green (Proc. Soc. Exper. Biol. and Med., 61:113–115, 1946 *et seq.*) that an antiserum made from immunized rabbits will inactivate mammary cancer cells *in vitro*. It was not clear in this work that the observed antigenic differences were characteristic of the malignant tissue (or mammary tumor agent) *per se* since control tissues used were histologically normal and thus not strictly comparable. Also, the animals used as source of normal control material were not genetically identical with those used as source of tumor material.

We have used as source of antigen normal lactating mammary glands of strain dba mice, subline 1 with and without the mammary tumor agent. Three transplantable mammary tumors 15091A (Strain A), dbr B (Strain dbs), and L916 (strain C3H) were used as a source of cancer cell suspensions.

Serums were prepared by immunizing groups of 3 or more rabbits by multiple injections of filtrates of lactating mammary gland tissue or of a 6 per cent solution of spontaneous mammary adeno-carcinomata.

For test of cytotoxic activity of serums one volume of cancer cell suspension was mixed with 5 volumes of antiserum and incubated at 37° C. for 3 hours. Test mice were inoculated with 0.5 cc. of the mixture.

A definite inhibiting effect was observed for antiserums obtained with lactating mammary glands (dba) whether or not the mammary tumor agent was present. No inhibiting effect was observed from antiserum produced by multiple inoculations of spontaneous mammary adenocarcinomata filtrates arising in another strain of mice (C3H).

CYTOGENESIS OF PULMONARY TUMORS INDUCED BY URETHANE. F. K. MOSTOFI (by invitation) and C. D. LARSEN. (National Cancer Institute, Bethesda 14, Md.)

The earliest morphologic changes preceding and accompanying the development of pulmonary tumors induced by oral administration of urethane have been studied in mice. One hundred and fifty strain A male and female mice, 2½ months old, were given drinking water containing 0.1 per cent urethane for 13 weeks. Each week during this period 3 male and 3 female experimental and an equal number of control animals were autopsied. Study of sections of the lungs has yielded information regarding the controversy as to whether these tumors originate in the bronchi or alveoli and whether oncogenesis was preceded by inflammation or atelectasis. It is concluded that these tumors originate in the cells of alveolar lining and are not preceded by atelectasis, by changes in bronchial epithelium, or by inflammation. Concomitant changes in gonads and lesions of the kidneys are also described.

PRENATAL STIMULATION TO NEOPLASTIC GROWTH: LUNG ADENOMAS IN SUCKLING MICE AFTER URETHANE INJECTIONS DURING PREGNANCY. WILLIAM E. SMITH and PEYTON ROUS. (Laboratories of the Rockefeller Institute for Medical Research, New York, N.Y.)

Tumors quickly arise from fragments of the skin, stomach, and lungs of C strain mouse embryos implanted in adults, together with droplets of olive oil containing methylcholanthrene. Pulmonary adenomas sometimes form within 3 weeks. Tests have now shown that subcutaneous injections of urethane into pregnant C mice hasten the occurrence and increase the number of adenomas in their offspring.[1] Such tumors were present in 21 of 80 mice 60 to 70 days old from urethanized mothers, whereas there were none in 84

* Work done at Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, under a grant from the National Advisory Cancer Council.

[1] An unwitting extension of the findings of C. D. Larsen in Strain A mice examined 6 months after birth (personal communication; paper in press).

controls after 60 to 100 days. Control mice kept longer seldom showed them until after almost a year.

The lungs of many fetuses and sucklings were searched in serial section to learn when the adenomas began. The repeated urethane injections caused no recognizable growths in the fetal pulmonary tissue, nor any inflammation then or later. Adenomas became visible, tiny yet distinct, 3 days after birth, 9 to 13 days after the first of 4 to 6 anesthetizing urethane injections of the mother on successive days. They grew rapidly, often showing mitoses, and they were many times larger and wholly typical within 10 days. Serial sections of control lungs showed none.

COMPARATIVE SUSCEPTIBILITY OF THE LYMPHOID TISSUES OF STRAIN C57 BLACK MICE TO THE INDUCTION OF LYMPHOID TUMORS BY IRRADIATION. HENRY S. KAPLAN. (National Cancer Institute, Bethesda 14, Md.)

Young mice of strain C57 black were sacrificed at successive intervals after fractional whole-body irradiation (1000r) and the lymph nodes, spleens, and thymus glands were subjected to gross and histological examination and to bio-assay. Fifteen of 28 mice (53.6 per cent) developed lymphoid tumors with 57 to 135 days after the beginning of irradiation. Most of the tumors were confined to the thymus gland (in 4 instances to just 1 lobe) and in no case had the disease spread outside of the thorax. The only positive bio-assays arose from three thymic tissue fragments, one of which came from a mouse that revealed no histologic evidence of a lymphoma.

It is evident that lymphoid tumors occurring in irradiated young mice of this strain regularly make their first appearance in the thymus gland and secondarily spread to the mediastinum and lungs before invading distant lymphoid structures. It is therefore concluded that such lymphoid tumors are initially monocentric in origin, differing biologically from other neoplastic processes chiefly in their greater ability to disseminate and to invade the peripheral blood. Some of the factors which appear to modify the relative susceptibility of the lymphoid tissues to leukemogenic agents are discussed.

COMPARATIVE CARCINOGENIC EFFECTS BY X RADIATION AND P^{32}. AUSTIN M. BRUES, GEORGE A. SACHER (by invitation), MIRIAM P. FINKEL (by invitation), and HERMANN LISCO. (Argonne National Laboratory, Chicago 37, Ill.)

Production of lymphoma has been studied in CF1, female mice, using total body X-ray and injection of P^{32}. Control mice show an exponentially increasing susceptibility to the spontaneous disease, with doubling of the tumor expectancy about every 75 days, until 700 days of age. Beyond this time, the rate fails to increase further.

Using different patterns of X-ray treatment, it is found that 400r total body X-ray is more effective if divided over a ten-day period than if it is either given as single dose or divided over 40 days. Increasing doses are more effective until a saturation value is reached, which appears to be at a probability of approximately 0.01 lymphomas per mouse per day. This is about the same as the saturation value reached in old age (after 700 days). There is also evidence for a threshold dose or dose rate below which early tumors are not induced.

P^{32} is a comparable carcinogenic stimulus and, again, a threshold dose or dose rate is encountered. The rate of tumor production with both agents is roughly proportional to the respective lethal doses. P^{32} is effective, whether given in a single dose or in monthly divided doses.

A QUANTITATIVE DESCRIPTION OF CARCINOGENESIS BY ULTRAVIOLET RADIATION. HAROLD F. BLUM. (National Cancer Institute and Department of Biology, Princeton University, Princeton, N.J.)

A simple differential equation, based on the assumption that repeated doses of ultraviolet radiation progressively accelerate the rate of tumor cell proliferation, gives a good quantitative description of the results of experiments previously carried out by the writer and his co-workers. While this description does not completely fit all details of the data, the deviations can be accounted for without great difficulty. No other simple hypothesis which has been examined, including some that appear tacit in more comomnly accepted theories of carcinogenesis, describes these data with any degree of accuracy. More than one hypothesis regarding the intimate mechanism might be rationalized with this description, but, if the basic assumption of progressively accelerated cell-proliferation is correct, any successful theory of mechanism must be consistent therewith.

THE EFFECT OF INTERRUPTED APPLICATIONS OF CARCINOGENS ON THE FORMATION OF NEOPLASMS. H. P. RUSCH and B. E. KLINE. (McArdle Memorial Laboratory, Medical School, University of Wisconsin, Madison 6, Wisc.)

The rate of tumor formation resulting from the continuous treatment with carcinogens is well known, but the effect of applying the same amount of carcinogen agents over a period that is interrupted by varying periods of rest has not been thoroughly investigated. The skin of the backs of mice were painted for four months with a solution of methylcholanthrene. At the end of this period, the few animals that had developed papillomas were discarded and the rest of the mice were divided into several groups of forty mice. One group was given no further treatment and was kept as a control. Another group was painted on the skin with a solution of croton oil for a period of two months and this treatment started immediately after the cessation of the hydrocarbon applications. The other groups also were painted with croton oil but periods of one, two, and three months of rest were allowed before the croton oil was started. The rate of tumor formation and the ultimate incidence of tumors was the same when one

or two months elapsed between the applications of the hydrocarbon and the croton oil as it was when there was no intervening rest period. When three months intervened between the two forms of treatment, the rate of tumor formation was the same as the control group. Similar experiments in which ultraviolet irradiation was employed as the carcinogenic agent gave essentially the same results.

GASTRIC LESIONS PRODUCED IN RATS BY TRIBUTYRIN DIETS. W. D. SALMON and D. H. COPELAND. (Introduced by R. W. Engel.) (Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn, Ala.)

The stomachs of over 100 rats of the Alabama Experiment Station (AES) strain that received complete diets containing 15 to 25 per cent tributyrin for periods of 3 to 37 weeks have been examined.

In the gross the stomachs were enlarged and the external surface of the forestomachs had numerous irregular protuberances. In some cases well circumscribed white nodules projected from the surface. In 5 rats similar nodules were visible on the external surface of the glandular region of the stomach. The inside of the stomach revealed large papillomatous growths covered with keratin and occasional ulcerated areas and craters opening to the surface.

Microscopically the following pathologic changes were observed in the forestomach of all the rats: acanthosis, hyperkeratosis, papilloma, infiltration, and occasional ulceration. Numerous hyperplastic spurs of squamous epithelium were found to penetrate the muscularis mucosa. Lesions in the glandular part of the stomach were observed in only 9 rats. In 4 rats these were simple cysts lined with glandular epithelium. In 5 rats the lesions were proliferating gland elements deeply placed in the submucosa and even in the external muscle layer. These lesions were not extremely anaplastic and were fairly well differentiated.

It is probable that none of the lesions can be classed as definitely precancerous, but they appear of sufficient significance to justify longer term feeding experiments upon tributyrin diets. The papillomatosis was more severe and more consistent than that produced in this laboratory by the Pappenheimer type of diet.

THE INCIDENCE OF BENZPYRENE INDUCED SARCOMAS IN DIABETIC AND ALLOXAN REFRACTIVE RATS OF THREE STRAINS. W. F. DUNNING, M. R. CURTIS, and C. FRIEDGOOD (by invitation). (Department of Pathology, Wayne University, College of Medicine and the Detroit Instiute of Cancer Research, Detroit, Mich.)

A 5 per cent aqueous solution of alloxan monohydrate in doses varying from 100 to 200 mg. per kilo of body weight was injected intraperitoneally into 351 rats of 3 inbred strains. Of these 54 per cent died in coma within 48 hours, 23 per cent developed a persistent glycosurea and hyperglycemia and 23 per cent were refractive. The optimum dose for the production of a chronic diabetes varied from 125 mg. per kilo of body weight for rats of the most susceptible strain to 175 mg. for rats of the most resistant strain.

The 82 rats with chronic diabetes, 38 of the alloxan refractive, and 20 control rats were injected subcutaneously on both sides with 0.2 cc. of a 1 per cent solution of benzpyrene in paraffin. Among the rats which survived the minimum latent period of 80 days, 67 per cent of the diabetics, 97 per cent of the alloxan refractive, and 90 per cent of the controls developed sarcomas at the sites of injection in an average of 165 $\pm$ 4.4, 189 $\pm$ 4.6, and 184 $\pm$ 7.2 days, respectively. In one strain 100 per cent of the diabetic and alloxan refractive rats developed sarcomas in an average of 174 $\pm$ 8.9 and 141 $\pm$ 9.2 days, respectively. Sarcomas developed in 69 per cent of the diabetic, 93 per cent of the alloxan refractive and 100 per cent of the control rats of another strain in an average of 160 $\pm$ 4.4, 197 $\pm$ 5.7, and 157 $\pm$ 1.7 days, respectively. The diabetic rats of the third strain died too early for a comparable test.

Diabetes shortened the lives of the affected rats, but did not delay or prevent the onset of benzpyrene induced sarcomas.

RHODANESE ACTIVITY AND TUMOR METABOLISM. OTTO ROSENTHAL. (Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania, Philadelpha 4, Pa.)

Mendel, Rudney, and Browman recently suggested that the enzyme rhodanese, which forms thiocyanate from HCN and thiosulfate, prevented inhibition of the Pasteur reaction by small amounts of HCN produced in tissue metabolism. The authors thought that absencé of the enzyme was responsible for the high aerobic glycolysis of tumors and of normal tissues with a tumor-like energy metabolism.

We have studied the distribution of rhodanese in 26 types of normal tissues from rat, rabbit, mouse, dog, and man. Altogether 54 normal tissues from these species were examined in addition to diverse forms of neoplasms. The enzyme activity (μM thiocyanate/min./gram dry tissue at pH 7.3 and 20° C.) ranged from 4 to 600 in normal epithelial tissues and from 1.5 to 37 in normal mesenchymal tissues. Twenty-eight per cent of the tissues showed values lower than 10; 63 per cent fell below 30. There were pronounced species differences in the activity of homologous tissues. The tumor values ranged from 1 (mouse sarcoma) to 54 (human intestinal carcinoma).

Our results corroborate the experimental findings of the previous authors regarding the rhodanese deficiency of sarcomas and certain normal tissues which are supposed to display a "tumor-like" metabolism. On the other hand, several normal tissues, which do not show metabolic abnormalities, were found to be extremely poor in rhodanese while carcinomas contained fair amounts of this enzyme. There is thus no evidence that the pattern of the energy metabolism of normal and malignant tissues is related to the distribution of rhodanese.

GLYCOLYSIS IN TUMOR HOMOGENATES. G. A. LEPAGE. (McArdle Memorial Laboratory, University of Wisconsin Medical School, Madison 6, Wisc.)

A medium has been devised for the study of anaerobic glycolysis in tumor homogenates. Cofactors and substrates added include glucose, adenosine triphosphate, diphosphopyridine nucleotide, bicarbonate, nicotinamide, magnesium, hexosediphosphate, pyruvic acid, and fluoride. The optimal levels for each were determined. The medium permits esterification of inorganic phosphorus. The homogenates metabolize glucose and maintain the phosphate bond energy for relatively long periods.

OXALACETATE METABOLISM IN GLYCOLYZING TUMOR HOMOGENATES. VAN R. POTTER, G. A. LEPAGE and A. B. PARDEE (by invitation). (McArdle Memorial Laboratory, Medical School, University of Wisconsin, Madison 6, Wisc.)

It has previously been reported that oxalacetate could not be oxidized by tumor homogenates in reaction mixtures in which various normal tissues were able to oxidize oxalacetate through the various steps of the Krebs cycle. Since this oxidation requires the maintenance of the adenosine triphosphate (ATP) reservoir, and since the oxidations in the normal tissues tend to maintain ATP, it was of interest to follow the metabolism of oxalacetate by tumor homogenates in which the ATP was simultaneously being maintained by glycolysis. The reaction has been studied under both aerobic and anaerobic conditions. The reactions have been followed by means of the measurement of CO_2 output in the Warburg respirometer, as well as by the chemical determination of lactic acid, keto acids, and inorganic phosphate. The results obtained have been essentially the same under both aerobic and anaerobic conditions. Although oxalacetic acid disappeared in glycolyzing reaction mixtures in the presence of fluoride, an equivalent amount of lactic acid did not form, while the CO_2 output was the same. The data therefore suggest that the oxalacetic acid was reduced, rather than oxidized. The results in O_2 and in N_2 are further support for this conclusion.

SERUM ZYMOHEXASE IN CANCER. JOHN A. SIBLEY and ALBERT L. LEHNINGER. (Introduced by C. Huggins.) (Department of Surgery, University of Chicago, Chicago 37, Ill.)

The enzyme zymohexase plays a basic role in glycolysis and hence in tumor metabolism, and alteration in its serum level in tumor-bearing rats has already been noted by Warburg and Christian.[1] In this paper a new method for the accurate assay of zymohexase based on the colorimetric determination of triose-phosphate is presented. In rats bearing Sarcoma 39 or Sarcoma 256 marked elevation in serum zymohexase occurs, the level being proportional to the amount of viable tumor tissue. Pregnancy or the production of emaciation, anemia, or infection as seen in tumor-bearing rats does not result in these high levels. Extirpation of the tumor produces a fall to normal levels, with subsequent rise if tumor recurs. An analysis of the tissues of normal and tumor-bearing rats shows no difference sufficient to account for the elevated serum level. The liver tumor animals is low while all other tissues tend to be somewhat higher than those of the normal rat. Tumor tissues have moderately high zymohexase content. The possible origin of excess serum zymohexase is discussed.

Serum zymohexase levels have been determined on normal humans and on patients with various diseases. Elevated levels are seen in some cases of muscle disease and in certain cases of cancer. The results, however, are not consistent enough to be of diagnostic value.

[1] Warburg, O. and Christian, W., Biochem. Z., 314, 399 (1943).

SERUM ESTERASES IN CANCER. S. H. MOULTON (by invitation) and C. HUGGINS. (University of Chicago, Chicago 37, Ill.)

Colorless nitrophenyl esters of propionic and butyric acids on hydrolysis liberate yellow color which is the basis of our colorimetric assay of esterases. There is a physiologic difference in the simple esterases splitting short chain fatty acids and activity against the long chains (lipase), the break in the series of substrates occurring sharply between 3 and 4 carbon fatty acids.

Transplantable sarcoma 39 in rats causes a prompt decrease of the common esterase of serum; only later when the tumors are large is lipase decreased. In a series of 29 patients with prostatic cancer, 13 had abnormally low values of serum esterase.

COMPARISON OF THE UPTAKE OF C^{14} FROM LABELED ALANINE AND GLYCINE INTO SLICES OF NORMAL LIVER AND HEPATOMA. P. C. ZAMECNIK, and (by invitation) I. D. FRANTZ, JR., R. B. LOFTFIELD, and M. L. STEPHENSON. (Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University, at the Massachusetts General Hospital; and the Radioactivity Center, Massachusetts Institute of Technology, Boston, Mass.)

dl-alanine and glycine were labeled in the carboxyl positions with C^{14}. Primary hepatomas were induced in rats of the Harvard colony by means of a butter-yellow diet. Normal livers and hepatoma nodules were removed and sliced at 0.5 mm. thickness by means of a Stadie slicer. Slices were incubated in Warburg flasks, in a medium consisting of Krebs-Ringer-phosphate, with the addition of either labeled dl-alanine or glycine. After $3\frac{1}{2}$ hours' incubation at 37° C., pH 7.4, in an atmosphere of 100 per cent oxygen, the slices were washed and homogenized. The protein was precipitated with trichloroacetic acid, and hydrolyzed with 6N hydrochloric acid. The ninhydrin procedure was used to liberate carbon dioxide from the carboxyl position of amino acids in the hydrolysate. This carbon dioxide

was trapped as barium carbonate, and the activity contained therein was determined by solid counting, using a thin windowed Geiger-Mueller counter.

Results of 7 experiments carried out with dl-alanine indicate that an average of six times as much radioactivity, per mole of amino acid present, is found in the protein hydrolysates of the hepatoma nodules as is found in the protein hydrolysates of normal livers. The values for the non-malignant portions of the livers bearing hepatomas were intermediate between the values for normal livers and hepatomas. In a single experiment carried out with glycine, approximately three times the concentration of activity was found in the hepatoma slices as was found in the normal liver slices. These experiments appear to indicate an increased rate of protein synthesis in the hepatomas as compared with the control livers.

EFFECT OF ADDED PHOSPHATE ON GLUTAMINE DESAMIDATION IN TUMORS. JESSE P. GREENSTEIN and FLORENCE M. LEUTHARDT (by invitation). (National Cancer Institute, Bethesda 14, Md.)

There appear to be at least two types of glutaminase in animal tissues, one noted in liver, brain, and spleen the activity of which is considerably accelerated by added inorganic phosphate, and the other found in kidney the activity of which is unaffected by added phosphate. Glutamine incubated with digests of primary rat hepatoma, and of the following transplanted mouse tumors, hepatoma, sarcoma, leukemia, amelanotic melanoma, and mammary tumor, is more rapidly desamidated when phosphate is added to the digests. According to the criteria used, this indicates that these tumors possess a glutaminase of the liver rather than of the kidney type.

NITROGEN METABOLISM OF MOUSE EPIDERMIS IN CARCINOGENESIS. II. UREA AND AMMONIA. EUGENE ROBERTS and SAM FRANKEL (by invitation). (Barnard Free Skin and Cancer Hospital, St. Louis 3, Mo.)

Extensive amino acid analyses have suggested that there is a decrease from normal in the rate of degradation of a number of amino acids in epidermis made hyperplastic with methylcholanthrene and in tumors derived therefrom.

The epidermis of the adult female Swiss mouse was found to have the high urea and ammonia contents of 167 and 32 mg. per cent (fresh weight basis) respectively. Three applications of methylcholanthrene in benzene resulted in decreases of urea to 88 mg. per cent and ammonia to 22 mg. per cent. The urea values were 43 and 61 mg. per cent and the ammonia values 9 and 13 mg. per cent, respectively, in two lines of transplantable squamous cell carcinomata derived independently from tumors induced by the application of carcinogen. In the non-carcinogenic hyperplasia produced by the application of benzene alone or of benzene containing 0.1 per cent croton oil there was a drop in urea to 106 and 88 mg. per cent but a significant increase in ammonia content over normal to 44 mg. per cent in both instances. No urease activity could be demonstrated in normal mouse epidermis. The results are tentatively interpreted to indicate that there is a decrease in the rate of the irreversible conversion of ammonia to urea but not in the production of ammonia from amino acids (proteins) in non-carcinogenic hyperplasia, while in carcinogenesis there is also a decrease in the rate of formation of ammonia. Other possibilities were discussed.

ADRENAL LIPIDS IN CANCER. W. R. BLOOR, FRANCES L. HAVEN and CHALLISS RANDALL (by invitation). (University of Rochester School of Medicine and Dentistry, Rochester, N.Y.)

The lipid content of blood and adrenals of rats bearing Walker 256 tumor has been investigated. In rats with large tumors the lipids of the blood were much higher than normal. The adrenals of these animals were lower than normal in percentage of steroids and higher than normal in fatty acid content. The decrease in steroids is partially compensated for by an enlargement of the adrenals.

CHANGES IN HEAT COAGULATION OF PLASMA IN MALIGNANT NEOPLASIA. MAURICE M. BLACK, HERMAN BOLKER and ISRAEL S. KLEINER. (Introduced by M. J. KOPAC.) (Brooklyn Cancer Institute, Brooklyn, N.Y., and New York Medical College, New York, N.Y.)

A ten second immersion of a plasma sample diluted 1:5 with distilled water in a boiling water bath produced turbidity which could be measured quantitatively in a photoelectric colorimeter. In an examination of 203 plasma samples from presumably healthy individuals 95 per cent of the readings fell below 70, 5 per cent were between 70 to 80, while less than 1 per cent were 80 or above. Similar testing of 101 plasma samples from individuals suffering from various types of malignancies disclosed that 25 per cent were below 70, 13 per cent between 70 to 80, while 62 per cent had values of 80 or above.

This series was also tested simultaneously by the reducing power method previously reported. Combination of the two techniques identified 89 per cent of the cancer cases. In no case was a control sample falsely diagnosed. However, increased turbidity readings have also been obtained in tuberculosis, active rheumatic fever, and periarteritis nodosa.

The turbidity appears to be related to the fibrinogen content of the plasma since: a. serum shows a decrease rather than an increase in turbidity by this method, and b. chemical determinations of fibronigen content tend to parallel the turbidity measurements.

Repeated testing of cancer cases under treatment revealed a tendency for the turbidity measurements to parallel the effect of therapy.

These preliminary observations warrant further study to evaluate their diagnostic and prognostic significance.

STUDIES ON CANCER DETECTION AND THERAPY: THE AFFINITY OF NEOPLASTIC, EMBRYONIC, AND TRAUMATIZED TISSUE FOR PORPHYRINS AND METALLOPORPHYRINS. FRANK H. J. FIGGE and GLENN S. WEILAND (by invitation). (Departments of Anatomy and Biochemistry, University of Maryland School of Medicine, Baltimore 1, Md.)

Studies on the cocarcinogenic action of porphyrins revealed that the sarcomas produced in mice by methylcholanthrene and porphyrin were red fluorescent. An attempt was made to determine the affinity of other types of neoplasia for porphyrins. Spontaneous and transplanted mammary carcinomas as well as the induced and transplanted sarcomas all concentrated the hematoporphyrin, which was injected intraperitoneally or subcutaneously at some distance from the tumor. Neoplastic tissues show a tendency to concentrate hematoporphyrin, protoporphyrin, deuteroporphyrin, mesoporphyrin, and coproporphyrin I. The margins of incisions and otherwise traumatized tissues became red fluorescent when hematoporphyrin was injected. Hematoporphyrin caused mouse embryos and placentas to become red fluorescent. In tumor-bearing mice as well as in normal mice, injected porphyrins also concentrated in lymph nodes and the greater omentum. These experiments indicated that growing tissues (both normal and neoplastic) have a strong affinity for porphyrins.

For some time, a partially successful attempt was made to utilize this phenomenon to increase the sensitivity of tumors to penetrating radiations. It was soon realized, however, that if the affinity of neoplastic tissues for porphyrins extended also to metalloporphyrins, then the porphyrins combined with various radioactive metals could be utilized for cancer detection and perhaps for therapy. It has been demonstrated that the introduction of a metal into the porphyrin molecule does not destroy the tendency of the porphyrin to concentrate in tumor tissues. The possibility of using radioactive metalloporphyrins for cancer detection and therapy appears promising and is being further investigated.

INDUCTION OF MALIGNANT TUMORS OF THE UTERUS AND UTERINE CERVIX IN MICE. S. C. PAN (by invitation) and W. U. GARDNER. (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

This is a preliminary report on the induction of uterine and cervical cancers in transplanted young adult uterine and cervical tissue with the carcinogenic hydrocarbon, methylcholanthrene. Ninety-eight mice of both inbred strains and hybrid stocks have been used in this investigation. Uterine cervices and horns of virgin mice into which small crystals of methylcholanthrene were inserted were transplanted into homologous hosts, usually subcutaneously.

Of the 98 mice, 31 up to this time have had carcinomas in the transplanted areas. Twenty-eight animals had epidermoid carcinomas, arising mostly from the cervix and occasionally from the horns, and 3 animals had adenocanthomas or adenocarcinomas arising from the transplanted horns. The transplants persisted in a higher per cent of mice of the A strain and the incidence of tumors was higher. The malignant tumors arose from the transplanted tissue as determined by examination of material obtained shortly after transplantation. Malignant tumors of connective tissue origin have also been observed, and histologically were seen to originate from the stroma of the endometrium.

The induced tumor first appeared at the site of transplantation from 2 or more weeks after the grafts were made and reached 2 to 2.5 cm. in size in a course of 2 to 3 months.

The induced tumors have been transplanted into other hosts and histologically have been shown to be identical with the original tumors.

UTERINE AND CERVICAL TUMORS IN UNTREATED AND HORMONE-TREATED MICE. W. U. GARDNER and S. C. PAN (by invitation). (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

Malignant tumors of the uterus and vagina developed in untreated mice of the "PM" stock. Thirteen spontaneous tumors of the uterus or vagina have been observed in 56 female mice that survived 200 days or more. Five of the tumors were epidermoid carcinomas, 7 were malignant tumors of undifferentiated cells, probably of epithelial origin, and one was a spindle cell sarcoma. Mice of this stock showed low fertility and two females had incompletely developed vaginas. The fertility decreased with continued inbreeding and the incidence of tumors increased.

Carcinomas of the uterine cervix and vagina also occurred in hybrid mice (PM × C3H) given estrogen and androgen but none appeared in untreated hybrids. Four of 10 estrogen-treated hybrid mice (C3H ♀ × PM ♂) that tolerated the treatment more than 200 days had carcinomas of uterine cervix or vagina. Nine carcinomas or invasive epithelial lesions were observed among 25 treated hybrids (PM ♀ × C3H ♂) that survived 200 days or more. All the thirteen tumors were epidermoid or invasive epithelial lesions that are considered to be early stages in tumor formation. None of the 82 mice in the control groups had carcinomas of the uterus or vaginas although 5 had uterine fibromyomas. In association with the neoplastic change of the epithelium, chronic proliferative vascular lesions have been observed involving the parametrium and uterine vessels.

ADENOCARCINOMA OF THE UTERUS IN AN ENDOCRINE IMBALANCE FEMALE RAT. CARROLL A. PFEIFFER. (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

An endocrine imbalance is produced in a female rat when a testis from a littermate male is grafted into it at birth. The presence of the testis masculinizes the

hypophysis so that it is not capable of releasing luteinizing hormone in amounts sufficient to cause ovulation or luteinization of the ovarian follicles. Therefore, a balance exists between the gonadotrophic hormone and the estrogen produced by the ovary such that estrogen is released at a constant level throughout the life of the rat. This level is lower than that at full estrus. It has been reported that leiomyomata are produced in the uteri of such imbalance females (Pfeiffer, C. A., Cancer Research, 6:491, 1946). An adenocarcinoma of the uterus has now also been produced under the conditions which exist in these imbalance animals. The animal bearing the tumor was 816 days of age. The tumor involved the entire left horn of the uterus and the ovary, forming a mass measuring 3.5 × 2.5 cm. It had involved the peritoneum and had been carried through the lymphatics to the region of the lesser curvature of the stomach. It had completely replaced the lymphoid tissue in the nodes of this area; each node was enlarged many times its normal size. It extended along the stomach and through the esophageal hiatus. It had metastasized to the lungs, the liver, and the spleen. The right horn of the uterus showed hyperplastic proliferation, which gave an indication of the underlying cause of the tumor formation.

AN OVARIAN TUMOR IN A HERMAPHRODITIC MOUSE. CHARLES W. HOOKER and LEONELL C. STRONG. (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

In the rarely encountered hermaphroditic mouse the specimens thus far described have had a testis on one side of the body and an ovary on the other side. In possessing both types of gonad from an early age, these animals are like the mice and rats in which Pfeiffer has produced an endocrine imbalance by transplanting testes to females at birth. An earlier hermaphrodite that we studied exhibited nearly all of the consequences of the experimental imbalance described by Pfeiffer. The present specimen was in all major respects identical with the earlier specimen with the exception that here the ovary was replaced by a tumor 23 mm. in diameter. The mass had undergone extensive necrosis, but the portion remaining appeared to be a granulosa-cell tumor. The experimentally produced imbalance is considered to be basically a disturbance of the pattern of release of hypophyseal gonadotrophins. If ovarian tumors in mice depend for their origin on an endocrine imbalance or appropriate change in hypophyseal activity, hermaphroditic mice might be expected to exhibit an increased incidence of ovarian tumors. Of seven hermaphroditic mice now listed in the literature one has had an ovarian tumor.

SOME STUDIES OF ESTROGEN—ANDROGEN ANTAGONISM. ROBERT A. HUSEBY and JOHN J. BITTNER. (Division of Cancer Biology, Dept. of Physiology, University of Minnesota Medical School, Minneapolis, Minn.)

Groups of $AXZF_1$ hybrid male mice were transplanted with vaginal fragments and two weeks later were injected with graded doses of estrone. On the day prior to the administration of the hormone, half of the animals were castrated. The hormone was administered in aqueous alcohol solution, each animal receiving two injections per day each day for three weeks. Within six hours after the last injection the animals were sacrificed and the vaginal grafts studied histologically and the mammary gland development studied by the whole mount technique.

The results of this study seem to indicate that for both the vaginal mucosa and the mammary epithelium, there exists an antagonism between testicular function and injected estrone since at certain dose levels no development was evident in these epithelia in the non-castrate animals while in the castrated animals receiving equivalent amounts of estrone, development of both vaginal mucosa and mammary glands was evident.

ENDOCRINE INTERRELATIONSHIP AND SPONTANEOUS TUMORS OF THE ADRENAL CORTEX IN NH MICE. MARTHELLA FRANTZ (by invitation), ARTHUR KIRSCHBAUM, and CARMEN CASAS (by invitation). (University of Minnesota Medical School, Minneapolis, Minn.)

The occurrence of spontaneous estrogen-secreting tumors of the adrenal cortex can be correlated with the early cessation of ovarian activity in the NH stock. NH mice become infertile at an earlier age than females of other stocks. Of 6 strains of mice studied, developing Graafian follicles were found in the ovaries of all except the NH at one year of age.

The vaginal secretion of mice with cortical adenomas is copious, watery, and high in cellular content. Epithelial cells and leukocytes are found in equal numbers. The ratio of cornified to non-cornified epithelial cells is 25 to 75.

When only the ovaries were removed from tumor-bearing mice, the vaginal smear picture was unaltered. When the adrenals alone were removed (ovaries remaining), then the vaginal smear became of castrate type within 5 days. This constitutes proof that the adrenal tumors were the primary source of estrogen.

The effect of gonadotrophic hormone was studied on the same mice (adrenalectomized or ovariectomized). No change could be elicited in the castrate smear of the adrenalectomized females, indicating the refractoriness of the ovaries to gonadotrophic hormone. The same amount of this hormone, when given to ovariectomized mice with adrenal tumors, induced a change in the vaginal smear picture. The quantity of secretion was increased, and the ratio of cornified to non-cornified cells increased from 25:75 to 50:50. The number of leukocytes remained the same. It would appear that gonadotrophic hormone enhanced estrogenic secretory activity of cortical adenomas.

IMPAIRMENT OF ESTROGEN-INDUCED TISSUE GROWTH IN THE CHICK GENITAL TRACT BY FOLIC ACID ANTAGONISTS. ROY HERTZ. (Introduced by H. B. Andervont). (National Cancer Institute, Bethesda, Md.)

Previous observations have indicated that the tissue growth response to estrogens in the genital tract is markedly reduced in the folic-acid-deficient chick. In addition, a direct quantitative relationship between the amount of folic acid ingested and the degree of estrogen response has been demonstrated.

In accordance with the Woods-Fildes principle of competitive metabolites, a number of folic acid analogues have been prepared which are potent inhibitors of the biological effects of folic acid. Data are presented concerning the comparative potency of several of these folic acid antagonists in inhibiting the tissue growth response to diethylstilbestrol in the chick.

These data demonstrate quantitative interference with hormone-induced tissue growth by nutritional means.

THE INHIBITION OF ANDROGEN-INDUCED COMB GROWTH IN THE CHICK BY METHYLCHOLANTHRENE. ROY HERTZ and WILLIAM TULLNER. (Introduced by H. B. Andervont). (National Cancer Institute, Bethesda, Md.)

The comb-growth response to maximal doses of subcutaneously administered androgen in female chicks was substantially retarded by the topical application of methylcholanthrene. After approximately 20 days of combined treatment, this retardation became less marked, and in approximately 20 per cent of the pullets gross abnormalities in comb formation appeared. Photographs of these anomalous combs were shown.

These effects were not associated with any general impairment of body growth. The data support the thesis that tissue-growth suppression may play a very important role in the initial phases of the response to chemical carcinogens.

ALTERATIONS OF PULMONARY AND OSSEOUS METASTASES IN PATIENTS WITH ADVANCED CANCER OF THE BREAST FOLLOWING HORMONAL THERAPY AND CASTRATION. I. T. NATHANSON and B. J. KENNEDY. (Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University and the Tumor Clinic of the Massachusetts General Hospital, Boston, Massachusetts and Pondville Hospital (Massachusetts Department of Public Health), Walpole, Mass.)

Demonstrable improvement has occurred following hormonal administration or deprivation in advanced carcinoma of the breast. Regression of primary tumors and recurrent nodules, epithelization of carcinomatous ulcers and decrease in size or disappearance of involved lymph nodes have been observed.

In patients with pulmonary lesions, regressions are manifested by a decrease in the size or disappearance of metastatic masses and reduction or elimination of pleural effusion. Osseous metastases have shown evidence of improvement by calcification of osteolytic lesions or tendencies to restoration of normal bone structure. These objective changes parallel subjective improvement.

Regression of both pulmonary and osseous lesions in pre-menopausal women may occur following castration or by the employment of large doses of androgenic hormone. Androgens also have produced calcification of osseous metastases in post-menopausal women. However, in some instances progression of osseous manifestations is observed in spite of subjective improvement. On the other hand, striking regressions of pulmonary metastases and calcification of osseous lesions following estrogen therapy have been observed primarily in post-menopausal women.

Thus, it appears that these similar effects following castration and androgenic and estrogenic administration are dependent to some extent upon the basic physiologic status of the host.

HISTOLOGICAL ALTERATIONS IN CANCERS OF THE BREAST IN PATIENTS TREATED WITH STEROID HORMONES. WILLIAM EMERSON (by invitation), B. J. KENNEDY (by invitation), and I. T. NATHANSON. (Medical Laboratories of the Collis P. Huntington Memorial Hospital, at the Massachusetts General Hospital, Boston, Mass.)

Regression of carcinoma of the breast has been observed following administration of hormonal preparations. The histological alterations in breast cancers during treatment were studied by means of biopsies and whole breast specimens. The most consistent change which occurred was replacement of tumor by fibrous tissue. The reaction was usually partial; cancer cells survived in most instances. In the early stages of regression, necrosis of tumor cells accompanied the fibrous proliferation. Plasma cells and lymphocytes frequently collected near necrotic foci. Whether the action of the drugs was primarily on the tumor cells or primarily on the stroma is not known.

Histochemical aspects of this problem were studied and were presented along with illustrations of the histological changes.

CHEMICAL AND SYSTEMIC CHANGES IN PATIENTS WITH ADVANCED CARCINOMA OF THE BREAST FOLLOWING HORMONAL THERAPY AND CASTRATION. B. J. KENNEDY (by invitation), DOROTHY M. TIBBETTS (by invitation), IRA T. NATHANSON, and JOSEPH C. AUB. (Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University at the Massachusetts General Hospital, Boston, Mass.)

Patients with advanced carcinoma of the breast have been castrated or received estrogenic or androgenic hormone therapy. The effects of these forms of therapy on the serum calcium, phosphorus, alkaline phosphatase, body weight, hemoglobin and red blood count have been studied. In addition, on some patients phosphorus, calcium, and nitrogen balance studies have been done. Certain changes in these factors have

been noted during treatment that can be correlated with the general response shown by the patient, as well as with X-ray changes of metastatic lesions.

The change of these factors by hormonal therapy in breast carcinoma has been compared to the changes of similar factors produced in the hormonal therapy or castration of patients with carcinoma of the prostate.

A summary of these studies was presented.

THE USE OF SEX HORMONES IN ADVANCED MAMMARY CARCINOMA IN FEMALE PATIENTS.* R. C. MELLORS (by invitation), G. C. ESCHER (by invitation), F. E. ADAIR, J. H. FARROW, H. Q. WOODARD (by invitation), and J. U. URBAN (by invitation). (The Sloan-Kettering Institute for Cancer Research of the Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York, N.Y.)

In the current study, 124 female patients with advanced inoperable or recurrent inoperable mammary carcinoma have been treated in the Breast Research Clinic of Memorial Hospital with either male or female sex hormones. Of this number, 82 have received treatment in excess of one month. This progress report deals with the clinical findings in this group of 82 patients.

Results with Male Sex Hormone.—Male sex hormone was administered as testosterone propionate,[1] 100 mg., intramuscularly, three times a week to 59 patients. The maximum cumulative dosage was 19,200 mg. Definite regression in size of cancer metastases occurred in 7 of 42 instances in bone, 2 of 20 in lung, 2 of 20 in lymph nodes, 2 of 12 in breast, 2 of 12 in skin, 1 of 8 in pleura, 2 of 4 in liver, and 1 of 1 in uterus. The cumulative dosage of testosterone propionate at the time of tumor regression varied from a minimum of 500 mg. to a maximum of 10,600 mg. The duration of regression extended over a range of 1 to 25 months, with approximately one half of the intervals extending 3 months or longer. Seven of the 12 patients with tumor regression were in the premenopausal age group. Regression of metastatic growth at one site and progression at another were observed in two instances. The incidence of symptomatic improvement greatly exceeded the frequency of tumor regression.

Results with Female Sex Hormone.—Female sex hormone was administered orally as diethyl stilbestrol,[2] 15 mg. daily in 16 instances; ethinyl estradiol,[3] 3 mg. daily in 12 instances; and estrone sulfate,[4] 30 mg. daily in 2 instances. The maximum cumulative dosage was 6720 mg. diethyl stilbestrol. Definite decrease in size of the primary mammary cancer occurred in 6 of 17 cases. Definite regression in growth of cutaneous metastases occurred in 4 of 13 cases. The cumulative dosage of estrogen at the time of tumor regression varied from a minimum of 420 to a maximum of 1800 mg. as diethyl stilbestrol. Six of the patients had received diethyl stilbestrol and 3 had received ethinyl estradiol. The duration of regression extended over a range of 1 to 12 months, with 6 periods of regression being 4 months or longer. The patients were all past the menopause, with 4 in the eighth decade of life. The incidence of symptomatic improvement exceeded the incidence of tumor regression.

* This work was supported by a Fellowship Grant of the American Cancer Society, recommended by the Committee on Growth of the National Research Council.

[1] Kindly supplied by the Schering Corporation.

[2] Kindly supplied by the Winthrop Corporation.

[3] Kindly supplied by the Schering Corporation.

[4] Kindly supplied by the Ayerst, McKenna, Harrison Company.

SOME PHYSIOLOGICAL EFFECTS OF CALORIE RESTRICTION AS RELATED TO THE RETARDATION OF TUMOR FORMATION. R. K. BOUTWELL, H. P. RUSCH, and MIRIAM K. BRUSH (by invitation). (McArdle Memorial Laboratory, Medical School, University of Wisconsin, Madison 6, Wisc.)

Since the incidence of many types of tumors is delayed or inhibited by calorie restriction, an understanding of the mechanism whereby calories exert their effect on tumor formation is desired. A systematic study of the weights of several organs of restricted and well fed mice was made. After periods of one week, one month, and 7 months on the experimental diets, mice were sacrificed in groups of 12 to 18 animals. The weight (as mg. per gram of body weight) of the thymus gland and the uterus was greater in the well fed animals, while the ratio of adrenal weight to body weight was greater in the restricted animals. There was little or no difference in the weight-ratio of the ovaries, thyroid, or pituitary to body weight between restricted and well fed animals.

These findings suggested that further criteria of the activity of the pituitary-adrenal cortical mechanism should be examined. A leukopenia and lymphopenia found in the restricted animals indicated enhanced cortical activity. The liver glycogen of restricted mice in contrast to full fed mice was 2 to 4 times as high during a major portion of the 24 hour day. However, in the morning just before feeding time, the liver glycogen level of the restricted mouse had declined to about 950 mg. per 100 gms.; less than 25 per cent of the level in mice fed *ad libitum*. The ascorbic acid content of the adrenals did not vary with calorie intake. The relation of these findings to carcinogenesis was discussed.

STUDIES ON RADIATION DEATH IN MICE. HENRY QUASTLER. (University of Illinois and Carle Foundation, Urbana, Ill.)

Death after irradiation occurs in a number of ways. Among the various types of reaction terminating in death, one of the best defined is acute radiation death, which occurs in mice in from about three to twelve days after a single exposure. The survival time is directly proportional to the weight and inversely proportional to the dose. An analogous expression holds for administration of daily doses if they are large enough to cause acute radiation death. The three-day-survival time which bounds the acute reaction is a re-

markably stable value; it occurs over a wide range of doses and depends very little on age and strain. However, with a split dose technique the process can be broken down into components.

GROWTH INHIBITION BY RADIATION IN BEANS. HENRY QUASTLER and MARIANNE BAER, WILSON N. STEWART, and ANN M. SCHERTIGER (by invitation). (University of Illinois and Carle Foundation, Urbana Ill.)

Irradiation inhibits growth in bean seedlings. The irradiated plants tend to fall into one of several well-defined classes rather than to present a picture of graded reaction. The reaction types can be related to components of the normal growth mechanism, and thus the analysis of radiation reactions can be used to study normal growth processes. By changing the doses and conditions of irradiation and by raying plants at various stages of development, the various types of reaction can be studied separately.

CUTANEOUS LEIOMYOMA OF GOLDFISH. HANS G. SCHLUMBERGER. (College of Medicine, Ohio State University, Columbus, Ohio)

In a large artificial pond about 1 to 2 per cent of the goldfish bear single or multiple orange-yellow tumors. The tumor-bearing fish average 20 cm. in length and from a study of their scales are approximately three to five years old. The tumors occur on the dorso-lateral surfaces of the trunk and/or on the fins; when multiple they are usually small, ranging in diameter from 2 to 10 mm. Occasionally one of these nodules may begin to grow rapidly and reach a size of 5 cm.; more often such a large tumor is the only lesion present. Histologically the neoplasms are composed of smooth muscle, probably derived from the arterioles of the corium. The cells of the small, slow growing lesions are well differentiated, but in the rapidly growing tumors the large cells with basophilic cytoplasm, vesicular nuclei, and prominent nucleoli suggest a malignant change. Visceral metastases have not been observed.

Both the "benign" and the "malignant" tumors show a similar pattern of growth in tissue culture. The cytoplasm is abundant, pseudopodia may be long; the nuclei are similar to those seen in tissue sections. Contractions were not observed and myofibrils were not demonstrated. In cultures kept for several weeks endothelial lined capillaries grew into the solid sheet of cells that surround the explant.

THE GENETICS AND PATHOLOGY OF THE MELANOMAS IN THE HYBRID OFFSPRING OF TWO SPECIES OF SWORDTAILS, *XIPHOPHORUS MONTEZUMAE* AND *XIPHOPHORUS HELLERII*. MYRON GORDON and R. F. NIGRELLI. (New York Aquarium, New York Zoological Society, New York, N.Y.)

The spotted caudal gene (*Sc*) of *X. montezumae*, when combined with *X. hellerii* modifying genes, produces melanomas under special genetic conditions. Melanomas appear in *Sc* hybrids, not in the first generation, but in some of the backcrosses to *X. hellerii* and in some of the second inbred generation. The tumor appears at the site of the original *Sc* pattern of *X. montezumae* that is, on the tail fin. Histologically, it is somewhat like the other melanotic growths in the tail fins reported for related hybrids. The corium is completely replaced by proliferating macromelanophores, which vary in size, shape and amount of melanin present, both within the same tumor and among similar tumors in different fish. The melanin-bearing cells at the periphery of the growth appear to be larger, show more numerous dendritic processes and are more loosely arranged than those towards the center of the tumor mass. There is considerable infiltration and destruction of muscle, bone and cartilage by these melanin-bearing cells. Numerous macrophages are invariably present at the periphery of the growth with an occasional one containing melanin. In some regions of the growth, the epithelium is thin and broken, probably as a result of the expanded corial growth. However, the epithelium in the region of the fins is often appreciably thickened. The tumor is especially characterized by extensive development of capillaries and sinuses together with numerous lacunae throughout but especially at the periphery of the growth.

MAST CELLS IN NEOPLASM. TALIA BALI. (Introduced by J. Furth). (Veterans Administration Hospital and Southwestern Medical College, Dallas, Texas)

The presence of mast cells in and about a tumor is a characteristic feature of the tumor. Of numerous neoplasms of mice studies, one (a luteoma) frequently contains large numbers of mast cells while another (a splenic reticulo-endothelioma) invariably contains them. All of 12 spontaneous and 4 transplanted tumors of the latter type examined were infiltrated with mast cells. Evidence is lacking that the mast cells are derived from the reticulo-endothelioma cells although they seem to arise *in situ* since the bone marrow is free from them. A much larger number of other neoplasms examined, including granulosa tumors and adenomas of the ovary, carcinomas and sarcomas of different sorts and leukemias, contained either no or only a rare mast cell although in the immediate or distant vicinity of some of these tumors mast cells were present in characteristic fashion.

NITROGEN MUSTARDS: THE RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND CHEMOTHERAPEUTIC ACTIVITY. J. H. BURCHENAL and J. B. RILEY. (Introduced by C. P. Rhoads). (The Sloan-Kettering Institute for Cancer Research and Memorial Hospital, New York, N.Y.)

In screening of 79 nitrogen mustard derivatives against transplanted leukemia in mice, certain definite correlations between chemical structure and chemotherapeutic activity, as demonstrated by a prolongation of survival time, have been noted. From these studies it appears that active compounds of this series have had at least two β halogenated alkyl groups. All

were secondary or tertiary amines. In addition to the bis β chloroethyl derivatives first studied, certain bis β chloropropyl analogs and combinations of β chloroethyl and β chloropropyl groups in the same molecule also were active. When bromine was substituted for chlorine the therapeutic effectiveness was retained. Diamino compounds having only a single β chloroethyl group on each nitrogen showed activity. Diamino, triamino, and tetramino derivatives having two β chloroethyl groups on each nitrogen atom also were chemotherapeutically active.

OBSERVATIONS ON THE IMPLANTATION OF VARIOUS TUMORS FROM HUMAN TISSUE ON THE CHORIOALLANTOIC MEMBRANE OF THE DEVELOPING CHICK EMBRYO. D. A. KARNOFSKY, L. M. RIDGWAY, P. A. PATTERSON. (Introduced by C. P. Rhoads). (The Sloan-Kettering Institute for Cancer Research and Memorial Hospital, New York, N.Y.)

A variety of tumors from human tissue has been implanted on the chorioallantoic membrane of the 8 to 10 day old chick embryo. Several different types of carcinomas and sarcomas have maintained themselves and shown evidence of mitotic activity. The tissue from normal lymph nodes and lymphosarcomas has produced an edematous reaction on the chorioallantoic membrane. The implantation of Hodgkin's disease tissue has frequently been associated with the formation of a generalized edema of the chick embryo. The cells of Hodgkin's disease, lymphosarcoma and normal lymphatic tissues rapidly degenerate on the membrane.

HISTOCHEMICAL OBSERVATIONS ON ALKALINE PHOSPHATASE IN HYPERPLASIA AND ADENOCARCINOMA OF HUMAN ENDOMETRIUM. S. B. GUSBERG and WILLIAM B. ATKINSON. (Introduced by E. T. Engle). (Department of Anatomy and of Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University, and The Sloane Hospital for Women, New York, N.Y.)

Recent studies have shown that endometrial alkaline phosphatase activity is regulated by estrogenic stimulation. The present work was designed to determine whether this relationship is present in abnormal endometrial growth. Alkaline phosphatase activity has been studied histochemically in a series of hyperplastic and carcinomatous human endometria.

In hyperplastic endometria alkaline phosphatase has been found in all cases. The amount and distribution varies without relation to either the degree of hyperplasia or to menopausal status of the patient. On the other hand, in adenocarcinoma a definite inverse relationship exists between the degree of tumor activity, as judged by the usual pathologic criteria, and the overall enzyme activity. In anaplastic tumors no evidence of phosphatase activity is present. As in the hyperplasias, tumors showing a definite tendency toward differentiation show a variable enzyme distribution and activity apparently unrelated to the menopausal status of the patient.

On the basis of these observations, it appears that the presence of alkaline phosphatase is not a nonspecific characteristic of tissue growth. The etiological role of estrogenic stimulation in endometrial hyperplasia may account for the mobilization of the enzyme seen in this condition. The disappearance of phosphatase in highly malignant tumors suggests that these tissues no longer respond to estrogen in this respect.

MITOCHONDRIA AND ASSOCIATED SUB-CELLULAR PARTICULATES IN MOUSE MELANOMAS. MARK WOODS (by invitation), H. G. DU BUY (by invitation), DEAN BURK, MARIE HESSELBACH, and MARY LACKEY (by invitation). (National Cancer Institute and Laboratory of Physical Biology, Bethesda 14, Md.)

Cloudman S-91 melanoma grown in dba mice contained three types of cells with respect to certain cytoplasmic particulates i.e., (a) predominantly, cells with melanin granules of variable melanin content, but no visible colorless mitochondria; and in grayish-white areas, (b) cells with colorless mitochondria, but no melanin granules, and (c) cells with both colorless mitochondria and granules of all gradations of melanin content. In the last two types the colorless mitochondria stained supravitally with Janus green B, and were of the same size range (roughly 0.5 to 1 μ) as the melanin granules. The melanin granules also stained with Janus green B, although this was less apparent when the granules were heavily melanized. The Algire amelanotic S91A melanoma, derived from the S-91 melanoma and grown in C mice, contained cells of type 2, the type that occurs in exclusively non-pigmented areas of the S-91 tumor. In some old amelanotic tumors, pigmented tumor cells occurred which contained fully melanotic granules, and other cells that contained granules of similar size but with much less melanin. Centrifugally isolated suspensions of S-91 melanin granules showed pronounced cytochrome oxidase, succinic dehydrogenase, and dopa oxidase activities. Similarly isolated amelanotic mitochondria from the S91A tumor showed the first two enzyme activities but not the last. Both types of particulates yielded, on alkaline hydrolysis, a material having an ultra violet absorption spectrum characteristic of nucleic acid and a positive orcin-HCl test for pentose. Phosphorus was also present. Melanin granules isolated from the Harding-Passey melanoma showed the same enzyme activities as the S-91 particulates. The available data are consistent with the view that the melanin granules in the mouse melanomas studied are modified mitochondria.

RETICULO-ENDOTHELIAL ACTIVITY IN MICE OF INBRED STRAINS. K. STERN. (Mount Sinai Medical Research Foundation, Chicago, Ill.)

An attempt was made to evaluate the storing ability of reticulo-endothelial tissue in two inbred mouse strains by means of intraperitoneal injections of carmine solutions. Varying concentrations and dosages of the dye were employed; the time interval between injection and sacrifice of animals ranged from 2 to 48

hours. Fifty animals of the C3H strain and 45 of the C57Bl strain were used for the experiments.

Paraffin sections (stained with hematoxylin-eosin and unstained) were prepared from liver, spleen, lung, kidney, skin and studied for quality and quantity of dye storage. Of the two strains examined, animals of the C3H strain showed a markedly weaker storing ability as compared with C57Bl mice. This was most clearly apparent in the liver where rough quantitative estimations of the Kupffer cells were carried out. In about 80 per cent of the C57Bl mice the number of dye-laden cells was considerably higher than the values obtained in C3H animals. Also, coarser and deeper staining carmine granules prevailed in the C57Bl mice.

Prolonged administration of a sulfonated scarlet red was found to result in proliferation of the Kupffer cells. In a preliminary experiment C57Bl animals showed a greater response of Kupffer cells to this stimulus than C3H mice. At present it is not possible to correlate the difference in reticulo-endothelial activity observed in the two strains with their well-known different susceptibility to spontaneous cancer development. Investigation of additional inbred strains will be required to establish, or reject, such a correlation.

THE ACTION OF CERTAIN MITOTIC POISONS ON TACTOID STRUCTURES. M. J. KOPAC. (Department of Biology, Washington Square College of Arts and Science, New York University and Laboratory of Experimental Cell Research, Marine Biological Laboratory, Woods Hole, Mass.)

The poisoning of mitotic processes by various chemical agents is well known. The activity of colchicine, for example, which blocks mitosis during the metaphase, is confined mainly to spindle structures. The organization of spindles by centrosomes or centromeres is frequently inhibited. Spindle fibrils, if already formed, may disintegrate on exposure to colchicine and similar agents.

Evidence provided by polarization optics supports the view that astral and spindle fibrils are cytoplasmic and, possibly, nuclear proteins organized into tactoid structures. Tactoids can be produced by Langmuir-Levine long range forces established between rod-shaped, sub-microscopic particles suspended in media of critical ionic strengths and pH. At certain concentrations and in proper ionic environments, crystalline tobacco mosaic virus nucleoprotein (TMV), because of its linear architecture, readily forms tactoids which show positive form birefringency.

The orientation of TMV particles at certain oil-water interfaces elicits an interfacial birefringence. Tactoid formation at various surface pressures can be studied with the drop-retraction and micro-tensiometer apparatus. A new procedure involving the transfer of an oil-water interface with its protein molecules from one aqueous environment to another permits an evaluation of the action of chemical agents on previously oriented TMV particles (tactoids). These methods, combined with polarization optics, provide a valuable tool for investigating the direct action of mitotic poisons on tactoids.

The action of colchicine, diphenylethylamine derivatives, and of flabone-amidines on the formation (a) and on the stability (b) of TMV tactoids at oil-water has been determined.

In (a), the TMV particles were exposed simultaneously to surface forces and to the mitotic poisons. The formation of tactoids by the influence of centrosomes or centromeres is thereby simulated by surface forces. In (b), the tactoids formed at oil-water interfaces were transferred to media containing the mitotic poison. Stability of such structures was indicated by persistent or non-persistent interfacial birefringency. This situation simulates the action of mitotic poisons on astral and spindle tactoids.

Although mitotic poisons, *per se*, have been disappointing in tumor chemotherapy, their continued study may augment our knowledge on mechanisms of cell division—a fundamental facet of the cancer problem.

CHANGES IN MITOSIS IN SARCOMA CELLS OF MICE TREATED WITH PODOPHYLLIN AND WITH COLCHICINE DERIVATIVES. ROSS C. MACCARDLE. (National Cancer Institute, Bethesda 14, Md.)

During a study of the capacity of chemical agents to damage tumor cells rapidly (which is being carried out by a group of investigators in the Chemotherapy Section), podophyllin was injected subcutaneously in single sublethal dose (20 micrograms per gram of body weight) into the contralateral axilla of mice bearing sarcoma 37 implanted in thigh muscles. The cytological findings are as follows. Within 2 to 16 hours after injection, podophyllin induced alteration of mitosis in various stages. In affected tumor cells, multipolar spindles and dissolution of preformed spindle fibers resulted in distorted chromosomal distribution, multinucleation and necrosis, as seen in Zenker-formol sections colored by Heidenhain's haematoxylin. Formed chromosomes were stained poorly in Feulgen preparations, while large nucleoli rich in nucleic acid remained abnormally intact. Chromosomes were swollen and vacuolated. Microincineration revealed marked increase of calcium and/or magnesium ash in achromatic figures.

In sarcoma cells of animals treated similarly with colchicine, no damage to already formed spindle fibers was observed; although in less than 24 hours after injection, spindle-formation appeared to be temporarily prevented (at 48 hours, mitosis was normal) while Feulgen-positive chromosomes remained in metaphase. After treatment with other compounds related to colchicine,—viz., colchicine, acetyliodocolchinol methyl ether, and trimethylcolchicinic acid—tumor cell-degeneration evidently eventuated in necrosis. Injured mitotic figures resembled those following podophyllin-treatment, excepting that chromosomes and cytoplasmic bodies were richly Feulgen-positive.

A COMPARISON OF THE TOXICITY AND PHARMACOLOGICAL ACTIONS OF PODOPHYLLOTOXIN AND PICROPODOPHYLLIN. MARGARET G. KELLY, E. WILLIAM LI-

GON, JR., and CLARKE DAVISON (all by invitation) and PAUL K. SMITH. (From the Department of Pharmacology, George Washington University School of Medicine, Washington 5, D.C.)

Podophyllotoxin and picropodophyllin are isomeric compounds. The former is carcinoclastic while, in the doses that have been used, the latter is without effect. The present study is a comparison of the pharmacological properties of the two compounds.

The median lethal doses of the two drugs were determined in normal white mice and in CAF_1 mice with sarcoma 37, administering the drugs by several routes. The ratio of the toxicity of podophyllotoxin to picropodophyllin is approximately 7 when given intraperitoneally and approximately 13 when given intravenously. Both drugs are approximately ten times as toxic in tumor mice as in normal mice. An investigation is in progress to determine whether this difference is due to a difference in the strains of mice used or to the presence of tumors in one group.

The responses of isolated rabbit duodenal strips were studied using drug concentrations of 0.5 to 10 mg. per liter. Much of the drugs did not go into solution at the higher concentrations. There were no marked changes in rhythmic movements although the strips always responded to pilocarpine.

Podophyllotoxin, 1 mg. per kg., and picropodophyllin, 7 mg. per kg., intraperitoneally, produce a 50 per cent drop in leukocyte count in one hour. The counts remain at this low level for several days. Similar results were obtained in animals given 3 doses at 2 day intervals. In these animals the counts were still down 10 days after the last dose of the drug but were normal after two weeks.

PLASMA MUCOPROTEIN LEVELS IN CANCER PATIENTS.

RICHARD J. WINZLER and JOHN W. MEHL, and IRENE M. SMYTH (by invitation). (Department of Biochemistry and Nutrition, University of Southern California School of Medicine, Los Angeles, Calif.)

Filtrates of plasma deproteinated with perchloric acid or sulfosalicylic acid contain a mixture of mucoproteins. These have been isolated and partially characterized, and a method for their quantitative estimation in plasma developed. Plasma proteins are removed by precipitation with 0.6 M perchloric acid, and the mucoproteins precipitated from the filtrate with 5 per cent phosphotungstic acid in 2 N HCl. The nitrogen, tyrosine, or carbohydrate content of the precipitate is determined, and the values expressed in milligrams per 100 ml. of plasma.

The determination has been applied to plasma from normal individuals, cancer patients and patients with pneumonia, tuberculosis or certain other diseases. The average mucroprotein-tyrosine level of 337 norma. plasmas was 2.7 ± 0.04 mg. per cent, whereas plasmas from 454 cancer patients averaged 6.1 ± 0.1 mg. per cent. The cancer patients were unselected cases, often relatively far advanced, and were in all cases diagnosed with certainty either by biopsy or at autopsy. Increases in plasma mucoprotein levels were also observed in pneumonia, tuberculosis, myocardial infarctions, and a number of other conditions.

Mucoproteins of similar nature have been shown to be present in circulating blood by carrying out electrophoresis experiments with pathological plasma at pH 4.5, and isolating and characterizing the small amounts of a component which migrated away from the other proteins toward the positive electrode.

That similar or identical mucoproteins increase in concentration in the plasma of individuals affected with disease of such different etiology suggests that some abnormality in protein metabolism is common to all of these conditions.

COLOR REACTIONS WITH MALIGNANT TUMORS.

EMIL WEISS. (Department of Pathology, Peoples Hospital, Chicago, Ill.)

Fresh unfixed tissue of malignant tumors ($\frac{1}{4}$–$\frac{1}{2}$ cc. suffice) is cut to small particles, placed in a tube, covered with 10 cc. of a saturated solution of litmus in 70 per cent acetone, corked and shaken vigorously for one minute. Malignant tissue turns the solution red while the control tube containing normal or benign tissue remains violet. A solution of 20 per cent salicylic acid in acetone, used in the same way, gives precipitation with malignant tumors and none with benign or normal tissues. As controls serve for each reaction: a) reagent control containing the reagent used in the test; b) positive control containing the reagent and a known malignant tissue; c) negative control containing the reagent and a known normal tissue. The controls are handled in the same way as the unknown. The test is interpreted as strong positive if salicylic acid and litmus give the described reactions for malignant tumors, as weak positive if only salicylic acid gives the typical reaction and as negative if precipitation does not occur with salicylic acid regardless of the litmus reaction. Impurities of proteins in the test tubes may cause false positive reactions while acids may cause false negative reactions. The test gave results corresponding with histological examinations in 95.17 per cent of malignant tumors (80.12 per cent strong positive, 15.06 per cent weak positive reactions). Benign tumors gave 4.24 per cent positive reactions (only weak positive). The test applies to all types of malignant tumors.

EFFECT OF THE INJECTION OF VARIOUS SUBSTANCES UPON THE *IN VIVO* ELECTRICAL RESISTANCE OF RATS.

HERBERT KAHLER and GEORGE BUCHANAN. (Introduced by H. B. Andervont). (National Cancer Institute, Bethesda 14, Md.)

The resistance of animal tissue *in vivo* was measured at 5,000 cycles. Intraperitoneal injection of massive doses of sugar was followed by an increase of body and tumor resistance. Other nonelectrolytes behaved similarly. Injection of sodium chloride had the opposite effect. Determinations were made upon the volume, electrical conductivity, glucose, and chloride concentration of the peritoneal fluid at successive time intervals

following glucose administration. These effects were related to shifts in body electrolytes and water. Intravenous injection of sugar was followed by an increase in resistance. From these experiments, it is concluded that the interstitial fluid conductivity is the chief component of the tissue contributing to these effects.

THE EFFECT OF NECROSIN ON A PARTICULAR TYPE OF TUMOR IN SWISS MICE.* VALY MENKIN. (From the Agnes Barr Chase Foundation for Cancer Research, Temple University School of Medicine, Philadelphia, Pa.)

A well-vascularized tumor, anaplastic in character for the most part, but with areas of adenomatous tendency occurs spontaneously in some Swiss mice. This epithelial tumor displays numerous mitotic figures and it metastasizes to the lungs. Duran-Reynals has demonstrated that proteins and dyes injected intravenously (1937, 1939), accumulate in tumors of experimental mice. He attributed this localization to the greater permeability of capillaries in such tumors. The writer (1943, 1946) has shown that the pattern of injury in inflammation is referable to a toxic substance in the euglobulin fraction of usually acid exudates of dogs. He has termed this toxic factor, necrosin. Necrosin contains some proteolytic activity.

The subcutaneous injection of necrosin, either after one or several administrations of the material in doses ranging from about 0.2 cc. to .05 cc. (diluted in the latter case with approximately five parts of saline) is soon followed in the great majority of cases by hemorrhagic extravasation or by the rupture of large hemorrhagic-like sinuses in the tumor itself. Necrosis of the tumor substance tends to follow this local hemorrhagic necrosis. The euglobulin of canine blood serum or the pseudoglobulin of canine exudates (LPF) fails to produce such effects. It is important to utilize an active preparation of necrosin, usually prepared from acid exudates. The injections of doses of necrosin, as used to date, tend to cause death of the animal. Severe damage to the liver cells is frequently found. These studies are being pursued further with the same and other types of tumors.

THE TRANSPLANTATION OF HETEROLOGOUS TUMORS BY INTRAVENOUS INOCULATION IN THE CHICK EMBRYO. DORIS H. BENDER, CHARLES FRIEDGOOD and HENRY F. LEE. (Introduced by Jonathan E. Rhoads). (Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania and the Children's Hospital of Philadelphia, Pa.)

In general, tissue transplanted from 1 species to another fails to grow. Successful transplantation has been accomplished under certain conditions such as by inoculation of neoplastic cells into the anterior chamber of the eye. It has also been accomplished by inoculation of such cells onto the chorioal antois or into the yolk sac of the chick embryo before complement appears in the blood.

Previous experience with intravenous inoculation of chick embryos with suspensions of *Mycobacterium tuberculosis* suggested this method might be useful in producing heterologous tumor growth and in learning whether a given tumor has a predilection for particular tissues within the chick embryo.

Chick embryos incubated for 11 days were prepared for intravenous injection by removal of the shell over the air sac and exposure of the allantoic veins by reflection of a portion of the shell membrane. Five hundredths cc. of tumor cell suspension was injected intravenously into each embryo. A total of 228 embryos were injected. Forty-four per cent of the embryos survived and were opened for examination on the twentieth day of incubation.

Following the intravenous inoculation of the chick embryo with suspensions of heterologous tumor cells, there was evidence of tumor growth in the brain or liver of 13 per cent of the surviving embryos.

In the experiments in which human tumor tissue was used, 10 per cent of survivors revealed histologic tumor "takes." In embryos surviving injection of a cell suspension prepared from the C57 strain mouse sarcoma 20 per cent showed tumor growth.

OBSERVATIONS ON ELECTROLYTE METABOLISM IN GASTRIC CANCER AND ADDISON'S DISEASE. AURELIA POTOR (by invitation), NELSON F. YOUNG (by invitation), F. HOMBURGER, and EDWARD C. REIFENSTEIN, JR. (Department of Clinical Investigation of The Sloan-Kettering Institute, Memorial Hospital Cancer Center, New York, N.Y.)

This report is the result of an attempt to obtain a more intimate knowledge of the relationships between the metabolic alterations in cancer and the role of the adrenal cortex secretions in these aberrations. Intensive studies were carried out on a normal man, a patient with gastric cancer, and a patient with Addison's disease. In each instance, sodium chloride was administered in progressively increasing amounts, in constant daily increments, over a period of ten days. Observation during this period included complete electrolyte balance studies and analyses of tissue removed at the termination of period of large salt intake.

The report presented graphical data describing details of the results of these studies. Linear representation of urinary excretion in the individuals observed shows divergence which appears to be significant; further studies are now in progress.

OBSERVATIONS ON A PARTICULATE BODY ASSOCIATED WITH EPITHELIAL CELLS CULTURED FROM MAMMARY CARCINOMAS OF MICE. KEITH R. PORTER and H. P. THOMPSON (by invitation). (Rockefeller Institute for Medical Research, New York 21, N.Y.)

Epithelial cells cultured from mammary tumors of high incidence C3H mice have been examined with the electron microscope. In preparations from half the tumors used (three out of six) an unusual particulate body was found associated with the cells. It is spherical

* Aided also by a grant from the National Advisory Cancer Council.

in shape and has a fairly uniform size and density. The unit particle appears in many cases to have a central dense portion and a less dense, capsule-like periphery. The average diameter of the central part is 75 μ while that of the outside is 135 μ. The particles occur singly, in pairs and in clusters of various sizes. They are found in all parts of the cell and frequently in large numbers on cell fragments. The same virus-like particle has not been encountered in other cultured tissue cells. Further evidence for or against its identification with the mammary tumor milk agent is being obtained from a study of cells from agent-free material.

A TRANSFORMATION OF NORMAL TO MALIGNANT TISSUE *IN VITRO*. KATHERINE SANFORD (by invitation), WILTON R. EARLE, E. L. SCHILLING (by invitation), EMILY DUCHESNE (by invitation), and EMMA SHELTON (by invitation). (National Cancer Institute, Bethesda 14, Md.)

In previous work on the action of 20-methylcholanthrene on normal mouse subcutaneous tissue fibroblasts, growing in a heterologous culture medium, the untreated control cells became sarcomatous. A new strain of C3H mouse fibroblasts from a milk-factor-free animal was started in tissue culture and has been maintained for over 3½ years in the same heterologous medium of chicken plasma, horse serum, chick embryo extract and isotonic saline. From the 143rd day in tissue culture until the 1324th day, seven sets of these cultures were injected intramuscularly into C3H strain mice. None of these 355 injections produced tumors. The 8th group of 60 injections from cells which had been 1382 days *in vitro*, produced 5 sarcomas at sites of injection. Two of these appeared within 30 days and the remaining 3 within 47 days after injection.

At the present time the cause of this transformation is not substantially clearer than with the earlier reported transformations. Three general possibilities must be considered: (a) that at least certain types of fibroblast cultures grown in such heterologous medium in tissue culture spontaneously go over to sarcoma cells; (b) that there is some unknown specific carcinogenic agent, either in the medium or otherwise affecting the culture; or (c) that this transformation has occurred due to accidental trace contamination of the cultures by the carcinogen 20-methylcholanthrene. In view of the care taken in handling, this last would appear a reasonable hypothesis only if such cultures were substantially more sensitive test objects for the carcinogenic action of this compound than are intact C3H strain mice.

PERFORATED CELLOPHANE AS A SOLID SUBSTRATE SUBSTITUTE FOR THE HETEROLOGOUS CLOT USED IN TISSUE CULTURE CANCER STUDIES. VIRGINIA J. EVANS, WILTON R. EARLE, and EMILY DUCHESNE, MARY-FRANCES EDWARD, ELIZABETH P. WILSON, GWENDOLYN LIKELY, and E. L. SCHILLING (by invitation). (National Cancer Institute, Bethesda 14, Md.)

Cellophane sheets as a solid substrate for the growth of tissue cells *in vitro* in Carrel flasks have been under study for nearly a year. Cultures of adult mouse fibroblasts, embryonic chick heart, and D, H, and L strains of Earle's sarcoma cells were used. The fluid culture medium was uniformly 40 per cent horse serum, 20 per cent (1:1) chick embryo extract and 40 per cent saline. Growth was compared when the cells were grown (a) on and under sheets of cellophane perforated with 1/32 inch holes on 3/32 inch centers; (b) on the glass surface of flasks; (c) in chicken plasma clots and (d) on and under sheets of unperforated cellophane. Growth on the glass surface of 3.5 cm. flasks and on unperforated cellophane was negligible; growth on perforated and under unperforated cellophane appeared at least the equal of those in the plasma matrix; those under perforated cellophane grew luxuriantly, reaching the periphery of the flask as a dense sheet in from 18 to 30 days. In 5 cm. diameter flasks the sheet reached the edge of the flask, within 19 to 32 days.

One set of wet weight determinations of strain L cultures in 5 cm. flasks under perforated cellophane at 39 days of age gave an average culture weight of 42.83 mg. Attempts to grow cultures in D 8.0 flasks have not as yet been satisfactory.

At least with normal fibroblasts, growth of cells under cellophane with other types of perforations indicates that finer, more closely spaced perforations allow even more satisfactory cultures due to closer adherence of the cells to the cellophane.

Applications of this work to cancer research were discussed.

CORRELATED DECREASES IN GLYCOLYSIS AND INOCULATION TAKE OF *IN VITRO*-INDUCED SARCOMA CELLS UPON PROLONGED SUBCULTURE. DEAN BURK, WILTON R. EARLE, MARIE L. HESSELBACH, CLARA E. FISCHER (by invitation), EMMA SHELTON (by invitation), and EDWARD L. SCHILLING. (National Cancer Institute, Bethesda 14, Md.)

Marked changed in metabolism and percentage effective inoculation have been noted among series of mouse tumor transplants newly derived from strains of mouse fibroblasts long-continued in tissue culture. The L, D, and H strains of Earle sarcomas, when newly derived from tissue cultures, showed considerably reduced glycolysis in the second to sixteenth transplant generations, as compared with these tumor strains when initiated several years before, and also to L and D strains in about the fifty-fifth to sixty-fifth transplant generation. The decrease in glycolysis was about 25 per cent at pH 7.3, the usual pH of measurement, but this comparative decrease was greatly enhanced when the measurements were made at pH 6.3, where the decrease attained 80 to 90 per cent. A correlated decrease in malignancy of these strains was also indicated by a notable reduction in the percentage of takes from tissue culture cells inoculated into mice. Between 1942 and 1945, the percentage of takes in

strains D, H, and L fell from 86, 74, and 70 to 50, 8, and 2 per cent, respectively.

Glycolysis of various Earle strain sarcoma tissue slices was found to be almost entirely eliminated within a few hours by exposure to pH 6.3. Return to pH 7.3, after increasing times of exposure to pH 6.3, resulted in decreased ability to recover, depending upon strain. Embryonic chick tissues had considerably less ability to withstand low pH (or recover from exposure thereto) than any of the tumor tissues studied. Anaerobic glycolysis was found (Earle strain H) to require neither CO_2 nor bicarbonate at any measurable concentration, and proceeded essentially as rapidly in phosphate medium as in CO_2-bicarbonate medium, comparable pH values being maintained.

REACTION OF CELLS TO X-RAYS, NITROGEN MUSTARD, AND EXTRACTS OF ADRENAL CORTEX *IN VITRO*. ROBERT SCHREK. (Tumor Research Unit, Hines Veterans Hospital, Hines, Ill.)

The toxicity of the reagents was tested by the method of unstained cell counts and was measured by the median effective dose. For cells from the rabbit thymus the median effective dose was 140r for x-rays, 0.46 gamma for nitrogen mustard HN2, 0.001 rat units for lipo-adrenal cortex (Upjohn) and 0.02 dog units for eschatin. The cells were relatively resistant to adrenocorticotropic hormone and to desoxycorticosterone.

Acid made the cells more resistant to x-rays but more sensitive to HN2. Incubation at 17° C. inhibited the action of x-rays and lipo-adrenal cortex but not the action of HN2.

Cells of bone marrow were sensitive to HN2 but were relatively resistant to x-rays and lipo-adrenal cortex. X-rays had a cytocidal action on cells from one half of the patients with lymphatic leukemia but had no action on cells of myelogenous leukemia. HN2 had an effect on cells of both lymphatic and myelogenous leukemia. Lipo-adrenal cortex had little or no effect on the cells of two patients with lymphatic leukemia.

Dark field illumination showed vacuoles in cells following irradiation but no vacuoles in cells killed with high doses of HN2. The nuclei of cells stained with safranin in the test tube were pyknotic after treatment with x-rays, but were leptochromatic after large doses of HN2. The findings suggest that the reaction of the cells to x-rays and lipo-adrenal cortex were similar in character but the reaction to nitrogen mustard was different.

STUDIES ON INVASIVENESS OF CANCER: ADHESIVENESS OF MALIGNANT CELLS IN VARIOUS HUMAN CANCERS. DALE REX COMAN and MORTON McCUTCHEON. (Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia, Pa.)

In previous studies on human squamous cell carcinomas, evidence was found indicating that the invasiveness of the cancer cells depended on their lessened adhesiveness. These studies have been extended to human cancers of glandular epithelium.

The adhesiveness of these cells as compared with that of normal cells was estimated by shaking tissues in a shaking device. The numbers of cells shaken loose were counted, and photographs of shaken and unshaken tissues were compared for cellularity and relative numbers of cells shaken out.

In 19 of 20 experiments relatively more cells were shaken out of cancerous than out of normal tissues.

This result justifies the conclusion that mutual adhesiveness of human carcinoma cells is less than that of corresponding normal cells, and supports the hypothesis that the invasive character of cancers is in part the result of decreased cellular adhesiveness.

GROWTH CHARACTERISTICS IN TISSUE CULTURE OF ANALOGOUS MOUSE MAMMARY CARCINOMAS AND THEIR RESPONSE TO RADIATION. ANNA GOLDFEDER and GLADYS CAMERON (by invitation). (Cancer Research Laboratory, Department of Hospitals, City of New York, and the Department of Biology, New York University, New York, N.Y.)

The mammary tumors used in these experiments were from two inbred strains of mice, dba and C3H, in both of which a high incidence of mammary tumors occurs. In a previous study by one of the authors (A.G.), it was found that implants of these two analogous tumors, in hosts of their respective strains, differed widely in their radiosensitivity. The latent periods of the irradiated implants of the respective tumors also varied significantly. For example, the longest latent period of an implant of the mammary tumor of the dba strain exposed to a threshold dose of 4500 r was 54 days, while that of the mammary tumor of the C3H strain exposed to a threshold dose of 2600 r was 38 days. It appears, therefore, that the ability of the tumor implants from the two strains of animals, to establish themselves in their respective hosts, and also their recuperation following radiation, are different. The question arises: Does the difference of these two analogous tumors in their rate of growth, and in their response to radiation depend upon the specific characteristics of the tumors, or does it depend upon the hosts?

In order to obviate the unknown factor present in the living animal, the present study, using the tissue culture method, was undertaken. The information obtained on the quality and growth characteristics of the tumor cells, irradiated and non-irradiated controls of both analogous mammary tumors grown in tissue culture, was illustrated and discussed.

INTRAVASCULAR AGGLUTINATION PHENOMENA ASSOCIATED WITH INTRAVENOUS INJECTION OF A POLYSACCHARIDE FROM *SERRATIA MARCESCENS*. J. S. YOUNGNER (by invitation) and GLEN H. ALGIRE. (National Cancer Institute, Bethesda 14, Md.)

Microscopic observations *in vivo* were made of circulation in normal and neoplastic tissues included within transparent chambers in mice, prior to and following intravenous injection of the polysaccharide.

Almost immediately after injection, numerous large, whitish masses were found in the circulating venous blood. These clumps persisted for about 10 minutes, then disappeared. Coincident with the disappearance of these masses, slowing of flow and appearance of intravascular agglutination of erythrocytes were noted. A decrease in functional capillaries in the striated muscle layer occurred, reaching a maximum in several hours and in some animals persisting for 48 to 72 hours. Return of capillary function to normal usually occurred 72 to 96 hours past injection.

Similar reactions followed intravenous injection of polysaccharide into mice bearing transplanted sarcomas. Although tumor vessels also responded by stasis and occlusion, this effect was minimal and hemorrhage and necrosis were not as severe as following intraperitoneal injection.

There is no indication at present of the relation between the intravascular occurrence of large whitish masses after intravenous injection of polysaccharide and events leading to decreased vascular supply to tissues. However, their possible role in occlusion of vessels and thrombus formation is suggested. These findings were discussed in relation to: (a) mechanism of action of bacterial products in producing hemorrhage and necrosis in tumors; (b) differences in effectiveness depending upon route of injection; (c) toxicity to the host.

EFFECTS OF *SERRATIA MARCESCENS* TUMOR-NECROTIZING POLYSACCHARIDE ON THE SPECIFIC GRAVITY OF MOUSE BLOOD AND PLASMA. DONALD BERKOWITZ (by invitation) and LYLE V. BECK. (From the Department of Physiology, Hahnemann Medical College, Philadelphia, Pa., and the National Cancer Institute, Bethesda 14, Md.)

Data obtained for CF mice, in determinations of the hematocrit value by centrifuging, and of whole blood and plasma specific gravities by the copper sulfate method, were shown.

A period of four hours between intraperitoneal injection of polysaccharide and sacrifice of the mice was chosen, since within this period the mice show marked symptoms of toxicity, i.e., diarrhea, prostration, death in some instances, and fall in body temperature and blood pressure. The relative amounts of polysaccharide injected into the tumor and nontumor-bearing mice respectively were those which previous experiments had suggested would produce roughly comparable toxic effects. Some of these toxic effects are indicative of shock, i.e., circulatory collapse. Nevertheless, in the present experiments there was no evidence for hemoconcentration, following administration of polysaccharide, except in obviously moribund mice.

The data indicate that administration of this polysaccharide produces an appreciable decrease in the specific gravity of the plasma, in both normal and tumor-bearing mice. Possible mechanisms for this effect were discussed.

ABSENCE OF EFFECT OF LYSED *T. CRUZI* PREPARATIONS ON SARCOMA 37. MORRIS BELKIN, ELEANOR J. TOBIE (by invitation), JOSEPHINE KAHLER (by invitation), and M. J. SHEAR. (National Cancer Institute and Division of Tropical Diseases, National Institute of Health, Bethesda 14, Md.)

In addition to the cooperative study with Hauschka *et al.* (J. Nat. Cancer Inst. 7: 189–197; 1947) independent experiments are being conducted at Bethesda on the effect of *Trypanosoma cruzi* on tumors. To date, lysed organisms from cultures of the following strains have been tested with negative results: Argentina, Brazil, Guatemala, Panama, Soule (used by Malisoff) and Wellcome (used by Roskin and Klyueva).

The trypanosomes were grown in flasks in a diphasic blood agar medium. After twelve days the organisms were harvested and pooled, then kept at 40° F. for about twenty-four hours to permit settling. The supernatant was then poured off, and the sedimented parasites lysed with distilled water. Metaphen was added in a final concentration of 1 to 10,000. Such preparations contained the lysed remains of about 285 million trypanosomes per cc.

The test tumor was sarcoma 37, transplanted intramuscularly in CAF_1 mice. Two types of experiments have been done: (a) effect within 48 hours of a single intraperitoneal injection of 1 cc. of a preparation containing about 285 million lysed organisms per cc.; (b) effect of about six daily intraperitoneal injections of the same dose. Toxicity in normal mice was also ascertained. Tumor controls were given equal amounts of diluted overlay of the culture medium.

No gross evidence was observed of any effect on the tumors by the lysed trypanosome preparations. Histologic studies revealed no significant difference between trypanosome-treated tumors and controls.

SUMMARY OF DATA IN FIRST SCREENING OF CHEMICAL AGENTS FOR POTENCY IN PRODUCING DAMAGE IN SARCOMA 37. M. J. SHEAR, JONATHAN L. HARTWELL (by invitation), VIRGINIA DOWNING (by invitation), ROSS C. MacCARDLE, JOSEPH LEITER (by invitation), ADRIAN PERRAULT (by invitation), and JAMES M. JOHNSON (by invitation). (National Cancer Institute, Bethesda 14, Md.)

The following changes have been made in the first screening technique: 15 tumor-bearing mice are used for each compound, instead of 8; instead of 1 control group, 3 groups each of 5 untreated tumor-bearing mice are killed at the same times (*viz.*, 8, 24 and 48 hours); in the cytological examination, reliance is placed upon Zenker-formol fixed, hematoxylin-eosin stained sections instead of an acetic-orcein smears; as before, a single subcutaneous injection of the maximum tolerated dose is employed.

The data on 318 compounds in 5 chemical categories show that most of them did not damage the tumors under these conditions. Positive results were as follows:

82 α, β-diphenylethylamines; 6 induced necrosis in S-37
87 quaternary ammonium salts; 8 induced necrosis in S-37
45 bis quaternary ammonium salts; 0 induced necrosis in S-37
41 acridines; 4 induced necrosis in S-37
63 arsenicals; 6 induced necrosis in S-37

Of the arsenicals, 5 of the 6 aliphatic derivatives were negative; all 32 of the aromatic pentavalent arsenicals were negative, whereas 5 of the 25 aromatic trivalent arsenicals induced tumor damage.

Further work is in progress with the compounds which yielded positive results in the first screening. Most of the above compounds have been further tested in repetition or extension of the first experiments; in all these instances the original positive findings have been confirmed.

Results with other types of chemical compounds will be reported at a later date.

STUDIES OF THE IMMUNOLOGICAL PROPERTIES OF *SERRATIA MARCESCENS* POLYSACCHARIDES. I. INFLUENCE OF IMMUNIZATION ON THE LETHAL ACTIVITY OF THE POLYSACCHARIDES. HUGH J. CREECH and DENIS R. A. WHARTON, EDWIN T. NISHIMURA and REED F. HANKWITZ, JR. (by invitation). (Lankenau Hospital Research Institute and the Institute for Cancer Research, Philadelphia 30, Pa.)

The polysaccharides obtained from two strains of *Serratia marcescens*, designated P-3 (G. S. strain and P-5 and P-10 (724 strain), have been found to be unrelated serologically. Injection of small amounts of the γ-globulin fraction of rabbit antisera toward the highly antigenic P-3 polysaccharide into both normal and tumor-bearing mice afforded pronounced protection against the lethal action of P-3 but did not significantly diminish the lethal action of the polysaccharides from the 724 strain. The γ-globulin fractions toward the less antigenic P-5 and P-10, at similar dosage levels, failed to protect the mice against death due to any of the polysaccharides. At high dosage levels, however, the γ-globulin fraction of antisera toward P-3 as well as P-5 and P-10 protected the mice against the lethal action of P-10. The rabbit protective antibodies toward the polysaccharides from different strains exhibit quantitative specificity. Qualitatively, at least, passive immunization does not prevent the tumor-necrotizing action of the polysaccharides.

Protection also can be elicited in normal and tumor-bearing mice by a single initial injection of one-tenth the lethal dose of polysaccharide. The high degree of protection, which was established within 24 hours and persisted for more than ten days, was primarily nonspecific, since all three polysaccharides effectively protected the mice against the action of lethal doses of the heterologous as well as the homologous polysaccharides. When the initial and lethal injections of polysaccharides were given more than ten days apart, however, strain specificity was observed.

STUDIES OF THE IMMUNOLOGICAL PROPERTIES OF *SERRATIA MARCESCENS* POLYSACCHARIDES. II. NATURE OF THE ANTIGENIC ACTION AND THE ANTIBODY RESPONSE. DENIS R. A. WHARTON (by invitation), HUGH J. CREECH and EDWIN T. NISHIMURA (by invitation). (Lankenau Hospital Research Institute and the Institute for Cancer Research, Philadelphia 30, Pa.)

B. prodigiosus polysaccharide can produce hemorrhagic and lethal effects in various animals. In sublethal doses, two effects have previously been noted: (a) it builds up a tolerance noticeable as early as the second or third day, and (b) complement-fixing, precipitating and agglutinating antibodies are demonstrable in rabbits and in man after a series of injections.

The present paper shows that following a *single* 100-γ injection of P-10 polysaccharide (1) agglutinating antibodies are demonstrable in the sera of mice, and (2) protective antibodies are demonstrable by 24 hours in mice. This protection reaches its height at the third day, when it is 90 to 100 per cent effective against a lethal dose of polysaccharide, declines sharply, and is gone by 15 to 21 days. Thus the protective antibody is maximal when agglutinating antibody is just definitely demonstrable, and is absent when the agglutinating antibody has attained a high titre. This indicates that there are at least two types of antibody to the polysaccharide complex. (c) Heating destroyes the hemorrhage-inducing toxic quality of the polysaccharide, but does not destroy its protective action. The polysaccharide acts like a true toxin. (d) Heating the polysaccharide does not destroy its antigenic activity *in vivo* or *in vitro* with respect to agglutinin production or precipitinogen activity. (e) Heating of the bacterial suspension, however, destroys most of the *in vitro* agglutinating potency against antipolysaccharide serum. (f) The tumor-necrotizing power of the polysaccharide is retained after heating, at least with large doses which produce marked destruction and are without hemorrhagic effect.

CHEMOTHERAPY OF LYMPHOMATA IN RELATION TO ADAPTATION. HERMAN BOLKER, MAURICE M. BLACK and ISRAEL S. KLEINER. (Introduced by M. J. Kopac). (Brooklyn Cancer Institute and New York Medical College, New York, N.Y.)

While x-radiation still appears to be the treatment of choice in Hodgkin's disease and lymphosarcoma, all too regularly the initial beneficial effects disappear as recurrence and insensitivity to treatment develops. In such circumstances the use of chemotherapeutic agents may prove of great value in the amelioration of symptoms and prolongation of life. The agents which we have used consist of two systems of drugs; the glycolytic inhibitors represented by sodium fluoride, iodoacetic acid and malonic acid, and an inhibitor of cytochrome-oxidase, sodium azide.

In a series of 11 cases of Hodgkin's disease and lymphosarcoma, all far advanced, 6 showed objective

evidence of beneficial effects of chemotherapy. The ability to reverse adaptation by alternate use of the chemotherapeutic agents was strikingly demonstrated in two of the cases. One case has undergone five induced remissions in a period of a year by the use of the glycolytic inhibitors, azide, nitrogen mustard and radiation. The reversal of adaptation to therapeutic agents appears possible in favorable cases by the judicious alteration of these drugs.

It is suggested that the glycolytic inhibitors and sodium azide be included in the armamentarium of therapy in the treatment of lymphomata.

THE EFFECT OF URETHANE ON SURVIVAL TIME IN TRANSPLANTED MOUSE LEUKEMIA. ARTHUR KIRSCHBAUM and CHEN-SHAN LU (by invitation). (University of Minnesota Medical School, Minneapolis, Minn.)

The effect of urethane on survival time has been tested on 5 transfer lines of F strain leukemia—3 myeloid and 2 lymphoid. Treatment was begun either at the time of transfer or after the development of the systemic disease.

The transfer lines of myeloid leukemia were affected more favorably than those of lymphoid disease. In the myeloid lines daily injection of 0.2 or 0.3 mg. per gram of body weight beginning at the time of transfer definitely delayed the onset of leukemia. In myeloid transfer line 686, 4 anesthetic doses, one on the day of transfer and one on each of the next 3 days, with no further treatment, effected significant delay (and in some cases possibly inhibition) of the development of leukemia. Survival time could be prolonged in both this transfer line and line 15-myeloid when treatment was begun even after the development of the systemic disease. Two anesthetic doses were given on successive days and then treatment was continued with daily doses of one-fifth and one-third the anesthetic dose. Line RE-myeloid was more malignant, and any prolongation of life by urethane was of doubtful significance. This was true also from the 2 lymphoid lines studied (291 and 86); treatment begun at the time of inoculation prolonged survival time only slightly in line 291 and not at all in line 86. The elevated white count of line 291 was readily depressed by urethane, even though the leukemia did not respond well from the standpoint of survival.

In both man and the mouse chronic myeloid leukemia appears to respond more favorably than other varieties of the disease.

STUDIES WITH ARSENIC[76]. WILLIAM NEAL, and LEON O. JACOBSON (by invitation), AUSTIN M. BRUES, and (by invitation) HOWARD DUCOFF, ROBERT STRAUBE, and THOMAS KELLY. (Argonne National Laboratory, and the Department of Medicine, University of Chicago, Chicago 37, Ill.)

Radioactive arsenic[76] has been used as a tracer in further studies of the metabolism of arsenic. Noted have been the early widespread distribution and rapid excretion in the laboratory animal and in man, and the altered distribution patterns in mice bearing implanted tumors. Arsenic[76] of high specific activity has been prepared by pile irradiation of cacodylic acid. Doses of 40 to 60 millicuries, calculated to give total body radiation of 25 to 50 roentgens, have been given clinically in therapeutic trials. Currently studied are the effects of this radiation on myelogenous leukemia, lymphatic leukemia, and Hodgkin's disease.

FOLIC ACID DERIVATIVES IN THE TREATMENT OF HUMAN LEUKEMIA. LEO M. MEYER, and (by invitation) HAROLD FINK, NORTON D. RITZ, MANUEL ROWEN, and ARTHUR SAWITZKY. (Department of Therapeutics, New York University Medical College, New York, N.Y.)

Derivatives of pteroyl glutamic acid (folic acid) were used in the treatment of patients with leukemia. Pteroyl triglutamic acid (fermentation 1 casei factor) produced no changes in the peripheral blood or bone marrow of cases with chronic leukemia. However, improvement in appetite and general well-being ensued. Two persons with acute lymphoblastic leukemia showed transient clinical and hematological remission. Seven patients with acute myeloid leukemia were unchanged. Three micro-biological antagonists to folic acid, namely pteroyl asportic acid, methyl pteroic acid and 4-amino pteroyl glutamic acid produced varying degrees of hematological and clinical changes in the order named.

Inconstant results were obtained with the first two compounds, probably due to inadequate dosage. No toxicity was observed. The third derivative (4-amino pteroyl glutamic acid) was found to be the most potent and was followed in all instances by a reduction in the leukocytes and immature cells in peripheral blood. Leukopenia and depression of the bone marrow occurred in some cases.

EFFECT OF ANTI-RETICULAR CYTOTOXIC SERUM ON THE BROWN-PEARCE CARCINOMA OF THE RABBIT. OTTO SAPHIR, and (by invitation) DAVID MOVITZ, and ALFRED STRAUSS. (Department of Pathology, Michael Reese Hospital, Chicago, Ill.)

The effect of spleen and marrow tissue antiserum on the Brown-Pearce carcinoma of the rabbit was investigated in nine experimental series. Each series consisted of 5 to 6 serum-treated and a similar number of controls, both groups being similar male hybrids. Anti-reticular cytotoxic serum may stimulate or depress the reticulo-endothelial system, depending upon dosage. As this system has been identified with resistance to tumor, the effect of the antiserum on the tumor would be dependent upon dosage. This was found almost consistently.

The serum was made by administering intravenously increasing quantities of normal spleen and marrow suspension-extract (saline) to a dog at 5-day intervals, until the complement-fixation titer was at least 1:80. In three series, with 0.03 cc., 0.09 cc., and 0.27 cc. given at 48-hour intervals, the malignancy of the Brown-

Pearce carcinoma was definitely enhanced, indicating a cytotoxic effect with depression of the reticulo-endothelial system.

Realizing the above dosage was cytotoxic, in the next three series only 0.00015 cc., 0.0003 cc., and 0.0009 cc. of the serum (approximately one one-hundredth of the cytotoxic dose) was administered. In one of these series an enhancement was still noticed, but in the other two no difference between serum-treated and controls occurred.

Thereupon, in the next three series the dosage was increased to one-tenth of that used in the first three series, shown to be cytotoxic. Furthermore, the dosage, 0.00125 cc., was given only at 7 to 10 day intervals, either two or three times. This resulted in a mild, but definitely tumor-inhibiting effect.

EFFECT OF AZO DYES ON LIVER PROTEINS. CLARK GRIFFIN, WILLIAM NYE, and J. MURRAY LUCK. (Introduced by J. C. Aub). (Department of Chemistry, Stanford University, Calif.)

The carcinogenic dye m′-methyl-p-dimethylaminoazobenzene was used in the induction of liver tumors in rats maintained on a basal purified ration. Groups of the animals were sacrificed at intervals, after perfusion with Ringer-Locke solution, and the liver proteins fractionated by the use of sodium chloride solutions and change of hydrogen-ion concentration. All fractionations were carried out at 1° ± 0.5° C.

Data were obtained from animals on the basal diet, and from others which received the azo dye supplement for periods of one, two, four, six and eight weeks. The values for ribonucleoprotein and desoxyribonucleoprotein in the normal rat were substantially equal to those reported in the literature. The administration of the azo dye resulted in a marked increase in the desoxyribonucleoprotein content which attained significance well in advance of the appearance of tumors. Determinations were also made of liver albumin, globulin, and flavoprotein.

FURTHER STUDIES ON THE PROTEIN-BOUND AMINOAZO DYES FORMED IN THE LIVERS OF RATS FED 4-DIMETHYLAMINOAZOBENZENE. E. C. MILLER and J. A. MILLER. (McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wisc.)

The livers of rats fed the hepatic carcinogen 4-dimethylaminoazobenzene contain aminoazo dyes chemically bound to the protein fraction. These dyes can be released only by prolonged alkaline or tryptic hydrolysis. The liberated dyes may be separated into polar and non-polar fractions. The former, which make up most of the total, have not been identified; the latter is composed of 4-monomethylaminoazobenzene and 4-aminoazobenzene.

Several correlations indicate that the bound dyes have an important place in the carcinogenic process induced by 4-dimethylaminoazobenzene. In the rat the bound dye was found only in the liver, the site of tumor induction. Low levels were found in the livers of mice, which develop tumors slowly; none was detected in the livers of 5 resistant species. The same levels of the same bound dyes were found in the livers of rats fed 4-dimethylaminoazobenzene or its carcinogenic metabolite 4-monomethylaminoazobenzene. Very low levels of different bound dyes were found when the non-carcinogenic metabolite 4-aminoazobenzene was fed. No bound dye was found in tumors arising in livers containing considerable levels of bound dye. When 4-dimethylaminoazobenzene was fed the level of bound dye reached a maximum after 3 to 4 weeks and thereafter slowly decreased. With a protective diet high in riboflavin the bound dye level was lower throughout the period of dye-feeding and the maximum was reached at 6 weeks. With the more active carcinogen 3′-methyl-4-dimethylaminoazobenzene the maximum was reached in 1½ weeks, while with the very weak carcinogen 4′-methyl-4-dimethylaminoazobenzene the maximum was not reached even by 22 weeks.

THE CARCINOGENICITY OF CERTAIN DERIVATIVES OF 4-DIMETHYLAMINOAZOBENZENE IN THE RAT. J. A. MILLER and E. C. MILLER. (McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wisc.)

Eighteen known or possible metabolites of the hepatic carcinogen 4-dimethylaminoazobenzene were tested for carcinogenic activity in the rat. Of these compounds only 4-monomethylaminoazobenzene, a known metabolite, proved to be active. Eight compounds, which appear to be metabolites of the dye, were inactive; 9 compounds which may possibly be metabolites also were inactive. A mixture of 9 known and possible metabolites was also found to be inactive. These data indicate that the primary carcinogen operative in tumor formation by 4-dimethylaminoazobenzene is probably an azo dye closely related to the parent carcinogen.

The carcinogenic activities of 19 other compounds related to 4-dimethylaminoazobenzene were tested to obtain more information on the structural feature needed for a 4-aminoazo dye to possess strong activity in the rat. Two conditions appear to be essential if the dye is to possess high activity: (a) At least one methyl group must be attached to the amino group together with the proper second substituent, and (b) the rings must bear either no substituents or carry only certain substituents, preferably in the 3′ position. The data on the carcinogenicity of the 2′-, 3′-, or 4′-methyl, chloro, and nitro derivatives of 4-dimethylaminoazobenzene indicate that the position of these groups determines the carcinogenicity of these compounds to a greater extent than does the type of group. The activity relationship was $3' > 2' > 4'$.

THE INTRACELLULAR DISTRIBUTION OF PROTEIN, NUCLEIC ACIDS, RIBOFLAVIN, AND PROTEIN-BOUND AMINOAZO DYE IN THE LIVERS OF RATS FED 4-DIMETHYLAMINOAZOBENZENE. J. M. PRICE, E. C. MILLER, and J. A. MILLER. (McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wisc.)

Rats were fed diets high and low in riboflavin with and without 4-dimethylaminoazobenzene for 4 weeks; at this time the maximal level of protein-bound dye is reached. The livers were homogenized in hypertonic sucrose solution and separated by differential centrifugation into nuclei, mitochondria, microsomes, and a final supernate. The whole homogenates and the fractions were analyzed for protein, nucleic acids, riboflavin, and protein-bound dye.

The analyses indicated that the ingestion of 4-dimethylaminoazobenzene reduced the protein content of the livers slightly through a decrease of about 35 per cent in the protein of the mitochondria. On the low riboflavin diet the protein of the nuclear fraction increased about 37 per cent, and the desoxypentose nucleic acid content increased about 31 per cent. Feeding the dye also produced decreases of about 40 per cent in the level of pentose nucleic acid in the mitochondria and the microsomes. The level of riboflavin in the liver was reduced by 28 to 43 per cent with the principal reductions occurring in the mitochondria and supernate. When 4-dimethylaminoazobenzene was fed protein-bound dye was found in all of the fractions, with the highest concentrations in the microsomes and supernate. A high level of dietary riboflavin lowered the level of protein-bound dye in each fraction.

CHANGES IN HEPATIC CYTOPLASMIC LIPIDS DURING CARBON TETRACHLORIDE INDUCTION OF HEPATOMA. N. KRETCHMER (by invitation), and C. P. BARNUM. (Department of Physiological Chemistry, University of Minnesota, Medical School, Minneapolis, Minn.)

The lipids of various portions of the cytoplasm were studied during carbon tetrachloride induction of hepatoma. Mice (C3H, males) were fed 0.1 ml. of an olive oil solution containing 40 per cent carbon tetrachloride over a period of 200 days at which time the hepatomas were fairly well developed. Fractions of cytoplasm were isolated by the method of differential centrifugation from normal liver, livers from mice fed for 60, 100, 150, and 200 days, and from the resultant hepatomas.

The large granules, microsomes and the final supernatant fluid were analyzed for total lipid, total phospholipid, fatty acids, and unsaponifiables. The fatty acids were analyzed for their unsaturation, and the phospholipids were analyzed for some of their constituent parts.

It was found that there is a progressive decrease in the unsaturation of the fatty acids in the large granule fraction, while the fatty acid unsaturation of the supernatant fluid tends to increase. During this period the microsome iodine value remained constant. In the hepatomata there is a marked decrease in the iodine value of the fatty acids in the various portions of the cytoplasm when they are compared to those of the final cirrhotic liver. Spectrophotometry of the fatty acids corroborated the iodine value data.

There are also correlative changes in constituent components of the phospholipids such as nitrogen, phosphorus and choline. These phospholipid analyses seem to indicate that a new type of lipid is entering the large granule and supernatant fluid lipid complex, namely, a a lipid which may possibly be of the cerebroside type.

HISTOCHEMICAL STUDIES OF ALKALINE PHOSPHATASE AT INTERVALS DURING CARCINOGENESIS IN RATS FED P-DIMETHYLAMINOAZOBENZENE. BJARNE PEARSON and THOMAS MORRIONE (by invitation). (Department of Pathology, College of Medicine, University of Vermont, Burlington, Vt.)

Seventy-seven male Sherman albino rats were placed on a synthetic diet plus p-dimethylaminoazobenzene and groups were taken monthly. At the end of four months the remaining rats were kept for an additional month on a stock diet minus the dye.

In the control animals the alkaline phosphatase is localized in the nuclei, nucleoli, nuclear and cell membranes of hepatic cells. The bile ducts and vascular endothelium appear more active than hepatic cells.

During the first month there is a proliferation of small bile ducts which can be seen distinctly as contrasted to the hepatic cells. During the second month there is a further increase in the proliferation of the cells which show intense alkaline phosphatase activity. In the third month there is also acinar formation. Cystadenomas, cholangiomas and hepatomas appear during the fourth month. The highest phosphatase activity in these tumors appears in small cells derived from bile ducts and endothelial lined spaces.

In the last group tumors composed of an admixture of hepatomas, cholangiomas, and cystadenomas are present. The alkaline phosphatase activity seems to be in proportion to the resemblance of the cells in the tumor to cells derived from the small proliferating bile ducts.

CHEMICAL CHANGES IN THE LIVER DURING P-DIMETHYLAMINOAZOBENZENE ADMINISTRATION. W. VAN B. ROBERTSON and NORMAN KRETCHMER (by invitation). (Departments of Experimental Medicine and Pathology, University of Vermont College of Medicine, Burlington, Vt.)

Rats (Sherman) were fed a synthetic diet containing p-dimethylaminoazobenzene for a period of 4 months at which time the carcinogen was removed and the tumors observed at that time were allowed to develop for another month. At monthly intervals the tumors were submitted to chemical analyses. The total, protein, lipid, acid soluble, and nucleic acid nitrogen and phosphorus and alkaline and acid phosphatase were determined.

Alkaline phosphatase progressively increased. The acid soluble fraction of the liver showed an increase after one month, which persisted. Nucleic acid, lipid, protein, and acid phosphatase dropped during the first month and then remained fairly constant at a decreased level.

The significance of these results was discussed.

THE INFLUENCE OF DIET ON THE ABILITY OF RAT LIVERS TO DESTROY N,N-DIMETHYL-P-AMINOAZOBENZENE AND RELATED COMPOUNDS *IN VITRO*. C. J. KENSLER. (Department of Pharmacology, Cornell University Medical College, and the Sloan-Kettering Institute for Cancer Research, New York 21, N.Y.)

The striking effect of diet on the incidence of hepatic tumors produced in the rat by the azo carcinogen N,N-dimethyl-p-aminoazobenzene (GMB) is well established. However, the mechanism of action of these dietary factors remains unknown.

It was reported from this laboratory that rat liver slices will destroy DMB and related compounds *in vitro*. During the course of a study of this destruction, it was observed that the livers from rats fed DMB on a high tumor incidence diet destroyed much less DMB than normal diet rats and that this change occurred even when the azo compound was omitted from this diet (brown rice—carrot). This change occurs quite rapidly, a decrease of 50 per cent or more occurring in 10 to 14 days. An examination of the effect of the addition of supplements such as yeast or riboflavin and casein, which will afford protection against hepatic tumor formation by DMB, indicates that these supplements maintain the ability of rat livers to destroy DMB at the same rate as normal livers. However, the addition of biotin to a rice-riboflavin-casein diet did not produce any decrease in ability to destroy DMB although under similar nutritional conditions biotin has been found to increase tumor incidence. Measurement of the riboflavin content of the livers, as well as the ability to destroy DMB, in rats fed eleven different diets, including the biotin diet, has shown that as the riboflavin content falls, so does the ability to destroy DMB. Biotin did not lower the riboflavin level in the liver in these experiments or in the earlier ones in which its procarcinogenic activity was observed.

THE EFFECT OF THIOSALICYLIC ACID, THIOURACIL, AND FOLIC ACID UPON P-DIMETHYLAMINOAZOBENZENE CARCINOGENESIS. PAUL N. HARRIS and G. H. A. CLOWES. (Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, Ind.)

Incorporation of thiosalicylic acid at the level of 1 per cent in a 10 per cent casein diet resulted in approximately two months retardation of liver tumor production by p-dimethylaminoazobenzene. The effect of addition of 1 per cent of thiosalicylic acid to a 20 per cent casein diet that in itself offered considerable retardation of p-dimethylaminoazobenzene carcinogenesis was even more striking, for of 14 rats that received the diet for 400 or more days, only one developed a liver tumor, and by this time tumors had appeared in one-third of the controls.

Thiouracil also was found to exert a protective effect when added at a level of $\frac{1}{2}$ per cent to these same two control diets. Urinary calculi were formed in some of the rats that received thiouracil.

Folic acid (pteroylglutamic acid) at the concentrations of 0.2 mg. and of 1.9 mg. per kilogram of 10 per cent casein diet had no effect upon tumor induction by p-dimethylaminoazobenzene.

FURTHER STUDIES ON MUTATIONS FROM METHYLCHOLANTHRENE-TREATED MICE. W. F. HOLLANDER (by invitation), and L. C. STRONG. (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

Young female mice of strains C3H, A, and JK were crossed with males of strains A, C3H, L, and JK. At about the time of conception the females were injected once intraperitoneally with 0.1 cc. of sesame oil containing 1.0 mg. of methylcholanthrene. Of the F_1 hybrid offspring produced, none showed mosaic genetic effects or induced tumors. All F_1 animals tested were apparently fully fertile, though several doubtful cases are still being tested. Approximately 5000 third-generation mice have been raised from the treated ancestry, and a majority (about 4000) have been autopsied at about weaning age. Of this generation a small number (about 0.5 per cent) have been abnormal, and several fairly definite mutant types have been obtained. The latter are (a) a growth defect—slow growth and temporarily bent and hooked tail; (b) deafness with slight waltzing tendency; (c) tendency to double hallux; (d) very dark agouti pelage; and (e) light belly color. These and others are still under breeding test.

THE EFFECT OF PARTIAL ABLATION OF THE BONE MARROW ON THE HEPATIC LESIONS PRODUCED BY 2-ACETAMINOFLUORENE. J. STASNEY, K. E. PASCHKIS and A. CANTAROW. (Jefferson Medical College, Philadelphia 7, Pa.)

Leukemia has been reported as one of the malignant processes induced in rats by feeding 2-acetaminofluorene. Inasmuch as partial ablation of the marrow has been reported to promote extramedullary hematopoiesis in the rabbit, 2-acetaminofluorene was fed to rats subjected to this procedure to observe its effect upon extramedullary hematopoietic foci and the incidence of leukemia. Large portions of the accessible marrow of male rats were destroyed and replaced by plastic and fiberglass. One group (ablation control) received a standard diet; another group received the same diet containing 0.03 per cent acetaminofluorene for 120 days and subsequently the diet without carcinogen. A third group received the diet plus acetaminofluorene, without marrow ablation (AAF control). Blood counts were made at intervals and animals in each group were sacrificed after 31 to 300 days.

Permanent, marked leukocytosis with evidence of myeloid immaturity occurred in both ablation groups to approximately the same degree. Both also presented foci of extramedullary myelopoiesis of limited extent.

The only striking difference between the two ablation groups was in the liver, which, in the acetaminofluorene-treated rats, was enormously enlarged, containing cysts and neoplasms; in some it constituted

36 per cent of the body weight. No significant hepatic enlargement occurred in the control ablation group.

Comparison of the two acetaminofluorene-treated groups revealed that marrow ablation enormously accelerated and intensified the hepatic lesions (cysts, malignancy) induced by this agent.

CONSISTENT PRODUCTION OF RAT MAMMARY CARCINOMA BY FEEDING 2-ACETYLAMINOFLUORENE. R. W. ENGEL, and D. H. COPELAND (by invitation). (Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn, Alabama.)

In an initial experiment with four littermate weanling female rats, it was observed that the ingestion of a diet containing 0.03 per cent of 2-acetylaminofluorene produced a 100 per cent incidence of mammary carcinoma in 5 months.

The experiment was repeated with an additional 9 female rats from 2 different litters. Mammary carcinomas were present in all 4 females of one of these litters in 132 days and in 4 females in the other litters in 119 days. The remaining female in the experiment died during the third month of the experiment at which time no neoplasms had developed. Thus a 100 per cent incidence of mammary carcinoma was observed in 12 female weanling rats which received a diet containing 0.03 per cent 2-acetylaminofluorene for 3 to 5 months.

The animals used in these esperiments were of the Alabama Experiment Station (AES) Strain. The diet used was composed of 9 per cent of casein, 20 per cent of degerminated corn grits, 50.7 per cent of sucrose, 15 per cent of lard, 4 per cent of salts, 1 per cent of cod liver oil, 0.3 per cent of l-cystine, 0.03 per cent of 2-acetylaminofluorene, and was supplemented with thiamin, riboflavin, pyridoxine, calcium pantothenate, inositol, niacin, choline, and alphatocopheral.

American Association for Cancer Research, Inc.

39th Annual Meeting

Hotel Dennis, Atlantic City, New Jersey
March 12 and 13, 1948

Proceedings of Business Sessions

Minutes of the Meeting of the Board of Directors Held March 11, 1948

The Board of Directors met at 8:00 p.m. on March 11, 1948 in Committee Room D in the Hotel Dennis in Atlantic City, New Jersey. Present were Directors Aub, Doisy, Furth, Gardner, Huggins, Little, Shear, and Taylor, with President Bittner in the chair.

It was voted to dispense with the reading of the minutes of the last meeting, inasmuch as approval of the individual members of the Board had been given by mail.

Reports of Officers

The Treasurer reported that, in compliance with the directive of the Board at its last meeting, the various gifts made to the Association had been deposited as a savings account with the Union and New Haven Trust Company as a special Journal Fund. The acceptance of a gift of $30.00 in memory of Therese Douglas Mann was reported. This sum has been added to the Journal Fund, making a total of $81.00 in this Fund.

The Treasurer's annual report and the report of the Auditor, Dr. Arthur Kirschbaum, appointed by the President, were read and accepted.

The Secretary reported that the Honorary Members elected at the last meeting had accepted their honorary membership in the Association. They are Dr. E. C. Dodds, Dr. E. L. Kennaway, and Dr. A. Lacassagne. After brief discussion it was voted that a form for Honorary Members be designed and adopted.

The proposal of Dr. Michael B. Shimkin that local sections of the Association be established was reported. After discussion of the several aspects of the proposal, it was voted "That a small committee be appointed to bring in recommendations on the modification of the By-Laws, Article IV, Section 3, dealing with branches of the Association, to the end that the Association will be in position to authorize the establishment of local sections when local groups of members so desire and meet the qualifications adopted."

The Secretary reported that a member of the Association had addressed to him a vigorous objection to the present practice of signing the proxy ballots for new members of the Board of Directors, the point of the argument being that the present voting is not secret. In the discussion it was emphasized that although the voter's signature is required on a proxy ballot authorizing the Secretary to cast a vote, the signature need not necessarily be on the ballot itself. It was therefore voted that an envelope providing a place for the signature of the voting member be furnished for mailing the ballot to the Secretary, and that the envelope be destroyed by the Secretary when the ballot is opened.

The Secretary reported the results of the referendum regarding dues ordered by vote of the members of the Association at their last annual business meeting. The alternative possibilities proposed and the voting were:

"I favor increasing the annual dues of active members from $3.00 to $7.00 which will include a paid subscription to *Cancer Research*"..... 230.

"I am not in favor of increasing the dues of active members"..... 93.

Reports of Committees

Program Committee.—Chairman J. C. Aub reported that all of the papers submitted for presentation by the date set by his Committee were placed on the program except one paper dealing with treatment by surgery. Three papers were received late and were not placed on the program.

Nominating Committee.—Chairman W. U. Gardner reported that his Committee had nominated for members of the Board of Directors to serve until 1951: Drs. J. J. Bittner, C. W. Hooker, Charles Huggins, Balduin Lucké, Clara J. Lynch, G. B. Mider, M. J. Shear, and W. L. Simpson. These names were listed on the proxy ballots sent to the members of the Association by the Secretary. Count of the ballots showed that the largest numbers of votes were given candidates Bittner, Hooker, Huggins, and Lucké. The Secretary was instructed to cast one vote for the nominees chosen by the members. The new Directors were then declared elected.

Cash on deposit (Union & New Haven Trust Co.) April 30, 1947			$2,420.47	
Check outstanding, April 30, 1947			522.00	$1,898.47
Cash on deposit, Greenwich Savings Bank, April 30, 1947				644.65
Interest on Savings account to January 1, 1948				9.69
Journal Fund (Union and New Haven Trust Co.)				81.00
Receipts, April 30, 1947 to February 29, 1948				
Dues collected			1,708.03	
Gifts			30.00	
Subscriptions to *Cancer Research*			20.00	
Tickets for annual dinner (paid by check)			19.00	1,777.03
				$4,410.84
Disbursements, April 30, 1947 to February 29, 1948				
Secretarial assistance		$225.00		
Annual meeting, Chicago May 16 and 17, 1947				
Notices	$ 26.50			
Programs	115.00			
Rental on meeting rooms	75.00			
Microphones, outlets	34.00			
Signs	8.00			
Lantern operators	46.12			
Rental on typewriter	3.25			
Identification badges, cards, tickets	33.00			
Annual dinner (balance due)	30.98	371.85		
Postage		60.12		
Bank charges on Foreign checks		.89		
Refund on overpaid dues		2.00		
Telegrams		1.76		
Subscriptions to *Cancer Research*		20.00		
Express charges		1.08		
Rubber stamps		4.20		
Journal Fund (Union & New Haven Trust Co.)		81.00		
Printing—notices, nomination forms, ballots and referendum forms		79.31		
Printing—Letterhead, envelopes		52.89		
		$900.10		$4,100.84
Balance February 29, 1948				3,510.74
Dues receivable				613.00
				$4,123.74

CHARLES W. HOOKER
Secretary-Treasurer

I hereby certify that the accounts and vouchers in the American Association for Cancer Research, Inc., for the above recorded period, have been examined by me, and that the above are true statements of its financial operations and of its financial conditions as of February 29, 1948.

ARTHUR KIRSCHBAUM
Auditor for the Directors

Membership Committee.—Chairman Charles Huggins reported that the Association now had 558 active members, 8 honorary members, 5 emeritus members, and 3 contributing members. The deaths of two members were announced with profound regret. They were:

DR. JULES C. ABELS, June 13, 1947
DR. ROBERT G. GREEN, September 6, 1947.

The resignations of 10 members were accepted:

EUGENE M. BURKE, Buffalo, New York
PHILIP P. COHEN, Madison, Wisconsin
EDGAR L. FRAZELL, New York, New York
HARRY F. FRIEDMAN, Boston, Massachusetts
HILDA LEE GOLTZ, Buffalo, New York
DAVID S. D. JESSUP, New York, New York
ALSON R. KILGORE, San Francisco, California
DAVID MARINE, New York, New York
ROBERT R. NEWELL, San Francisco, California
ANNETTE E. STENSTROM, Minneapolis, Minnesota.

The nominations for active membership were presented. The election of 55 candidates was recommended. They were:

ALBERT, A., PH.D., M.D., Mayo Clinic, Rochester, Minn.
AUSTER, LIONEL SANDLER, M.D., 21 East 82nd St., New York, N.Y.
BISKIND, GERSON R., M.D., 2200 Post Road, San Francisco, Calif.
BISKIND, MORTON S., M.D., 55 East 86th Street, New York, N.Y.
BLACK, MAURICE M., M.D., Brooklyn Cancer Institute, Brooklyn, N.Y.
BREEDIS, CHARLES, M.D., University of Pennsylvania School of Medicine, Philadelphia, Pa.
BUCHER, NANCY L. R., M.D., 72 West Cedar Street, Boston, Mass.
BUTT, HUGH ROLAND, M.D., 102–110 Second Avenue, S.W., Rochester, Minn.

CAMIEL, MORTIMER RICHARD, M.D., Brooklyn Cancer Institute, Brooklyn, N.Y.

CHAIKOFF, I. L., PH.D., M.D., University of California Medical School, Berkeley, Calif.

CHIPPS, H. DAVIS, School of Medicine, University of Washington, Seattle, Wash.

CLARK, DWIGHT EDWIN, M.D., University of Chicago, 950 East 59th Street, Chicago, Ill.

COLLER, FREDERICK A., M.D., University Hospital, Ann Arbor, Mich.

COMFORT, MANDRED WHITSET, M.D., 102–110 Second Avenue, S.W., Rochester, Minn.

COPELAND, MURRAY MARCUS, M.D., Georgetown University Hospital, Washington, D.C.

DANIEL, ESTHER P., D.SC., National Cancer Institute, Bethesda, Md.

DOUNCE, ALEXANDER L., PH.D., University of Rochester School of Medicine and Dentistry, Rochester, N.Y.

ESCHENBRENNER, ALLEN B., M.D., National Cancer Institute, Bethesda, Md.

ESCHER, GEORGE CHARLES, M.D., 444 East 68th Street, New York, N.Y.

FARBER, SIDNEY, M.D., 300 Longwood Avenue, Boston, Mass.

FINKEL, MIRIAM P., PH.D., 6518 Dorchester, Chicago, Ill.

FREIRE, PAULO MELLO, M.D., Yale University School of Medicine, New Haven, Conn.

GAL, EMERY MARTIN, PH.D., 155 El Camino Real, Berkeley, Calif.

GLINOS, ANDRE DIMITRI, M.D., 19 Marborough Street, Boston, Mass.

GOMORI, GEORGE, M.D., PH.D., University of Chicago, Chicago, Ill.

GUSBERG, S. B., M.D., 180 Fort Washington Avenue, New York, N.Y.

HALL, BYRON E., M.D., Mayo Clinic, Rochester, Minn.

HELWIG, ELSON B., M.D., Army Institute of Pathology, Washington, D.C.

HERTZ, ROY, PH.D., M.D., National Cancer Institute, Bethesda, Md.

JACOBSON, LEON O., M.D., University of Chicago, Chicago, Ill.

KAHLER, HERBERT, PH.D., National Cancer Institute, Bethesda, Md.

KARNOFSKY, DAVID A., M.D., Sloan Kettering Institute, New York, N.Y.

KING, LESTER S., M.D., 836 Wellington Avenue, Chicago, Ill.

LEITER, JOSEPH, B.S., National Cancer Institute, Bethesda, Md.

MACULLA, ESTHER, PH.D., Yale University School of Medicine, 333 Cedar Street, New Haven, Conn.

MARCHETTI, ANDREW A., M.D., Georgetown University Hospital, Washington, D.C.

MASON, HAROLD L., PH.D., Mayo Clinic, Rochester, Minn.

MERWIN, RUTH M., PH.D., National Cancer Institute, Bethesda, Md.

MUELLER, GERALD C., M.D., University of Wisconsin, Madison, Wis.

PENN, HARRY S., M.D., 727 West 7th Street, Los Angeles, Calif.

PLAUT, JULES ALAN, M.D., 441 Harvard, Claremont, Calif.

PLATT, WILLIAM R., M.D., Norton Memorial Infirmary, Louisville, Ky.

RAY, FRANCIS EARL, D.SC., University of Cincinnati, Cincinnati, Ohio.

SANFORD, KATHERINE KOONTZ, PH.D., National Cancer Institute, Bethesda, Md.

SEGALOFF, ALBERT, M.D., 3503 Prytania Street, New Orleans, La.

SMITH, WILLIAM E., M.D., Sloan-Kettering Institute for Cancer Research, New York, N.Y.

SOBEL, HARRY, PH.D., Beth Israel Hospital, New York, N.Y.

SPURR, CHARLES L., M.D., University of Chicago, Chicago, Ill.

STOCK, CHARLES CHESTER, PH.D., Sloan-Kettering Institute for Cancer Research, New York, N.Y.

WALTERS, WALTMAN, M.D., Mayo Clinic, Rochester, Minn.

WILHEIM, ROBERT, M.D., Mount Sinai Hospital, Chicago, Ill.

WOODS, MARK W., PH.D., National Cancer Institute, Bethesda, Md.

WORLEY, LEONARD GEORGE, PH.D., Brooklyn College, Brooklyn, N.Y.

YOUNG, NELSON F., PH.D., Sloan-Kettering Institute for Cancer Research, New York, N.Y.

YOUNGNER, JULIUS STUART, SC.D., National Cancer Institute, Bethesda, Md.

It was voted "That the report of the Committee be accepted and their recommendations adopted." The nominees were then declared elected as recommended. Doctor Huggins next presented his Committee's recommendation that Dr. Vasant Ramji Khanolkar of Bombay, India be elected an Honorary Member. The recommendation was approved, and Doctor Khanolkar was declared elected Honorary Member.

Journal Committee.—Chairman M. J. Shear read the report of his committee. The report is given here:

> The year 1947 was a disappointing one for subscribers to "Cancer Research" and for authors, in view of the interruptions and delays in publication of the monthly numbers since last spring. It was also a most trying year for those who shared the responsibility for the management of the journal.
>
> *RESUME*
>
> General Bayne-Jones returned to civilian life in the middle of 1946 and resumed the post of Editor. The journal was printed by a reliable company in New Haven. Early in 1947, however, the ownership of the company changed hands without warning to the Association, and the printing establishment ceased operations. The printing plant closed down with our manuscripts in its possession and with a goodly number of them already set up in type for printing of galleys.
>
> Dr. Bayne-Jones made heroic efforts to salvage from this debacle what could be saved. It soon became clear that the printing operations would have to be transferred, immediately, to another establishment. Together with Dr. Schram, a quick

survey was made of available printing companies willing to accept the contract under the conditions set. A contract was then made with a small printing outfit in New Jersey which promised to get out the journal in acceptable fashion.

Unfortunately, this arrangement proved unsatisfactory, and the material was not processed by the printer as it should have been.

In the meantime, Dr. Bayne-Jones was appointed President of a large medical institution in New York, and was no longer in a position to serve as Editor. So the journal lost its experienced editor at a time when it had lost its printer and had acquired a new printer who was rapidly demonstrating his inability to do a satisfactory job.

At this crisis, events took a more fortunate turn. Dr. Balduin Lucké generously consented to become Editor, and took hold with skill and vigor. Notice was served on the New Jersey printer that the contract would be terminated with the issuance of the June 1947 number, and a new contract was arranged with an experienced establishment in Ann Arbor, Michigan to take over publication beginning with the July 1947 number. This printer has been getting out the American Journal of Pathology in excellent fashion.

The New Jersey printer now has completed the June 1947 number, his last job for "Cancer Research." Dr. Lucké has furnished the new printer with manuscripts for all the numbers from July through December 1947. These are being processed rapidly and are expected to be issued in rapid succession. The July number is now in the hands of subscribers. In addition, the Editor has been working on the first few numbers for 1948, and expects that the Journal will be gotten current in a few months.

MANAGERIAL SITUATION

When the present incumbent was appointed Chairman of the Journal Committee, he found four governing groups for "Cancer Research" with ill-defined and overlapping jurisdictions. This situation requires clarification and remedy.

In the first place, legal ownership of the journal resides in the Donner Foundation. Secondly, the real executive power has been exercised by the Advisory Board, composed of representatives of the foundations generously taking the financial responsibility for the operations and for the deficit. Although it is called an "Advisory Board" it has been, in fact, the executive body. The third managerial body is the Editorial Committee, of which Dr. James B. Murphy has been Chairman. Fourthly, and finally, the Association itself appointed a Journal Committee.

In view of this complicated arrangement, and in view of the fact that the Journal Committee had no clear-cut duties or powers, its Chairman recommended to the President of the Association that the Journal Committee either be abolished or strengthened. The Committee was recently strengthened with the appointment by the President of Dr. Balduin Lucké and Dr. Albert Tannenbaum. Dr. Bayne-Jones regretfully submitted his resignation from this Committee, in view of the heavy responsibilities in his new post.

The re-organized Committee was charged with the duty of analyzing the situation, and of bringing to the Association recommendations directed towards: (a) eventual assumption by the Association of legal ownership of its journal; and (b) immediate tightening by the Association of the managerial set-up so as to get the journal operating more effectively.

JOURNAL COMMITTEE MEETING

The newly-constituted Committee convened in Philadelphia on January 7, 1948. Present were Drs. Schram, Lucké, Tannenbaum, and Shear. The events of 1947, outlined in the foregoing part of this report, were reviewed in oral reports from Dr. Schram, the Chairman of the Advisory Board, and from Dr. Lucké, the Editor.

Dr. Schram provided figures giving the financial picture of "Cancer Research." The estimated costs for 1947 were approximately as follows:

Printing bill (including paper, printing, engraving, wrapping, and mailing) Approx.		$12,000
Editorial and Abstracts' Offices		6,350
Abstractors' fees		800
Business office		1,200
Expenses (approximate total)		$20,000
Income		
200 subscribers at $5.00		$ 1,000
700 subscribers at $7.00		4,900
From dues of 600 members		600
Contributions:		
Childs Funds	$5,500	
Anna Fuller Fund	2,000	
Donner Foundation	5,000	
Pardee Fund	1,000	$13,500
TOTAL INCOME		$20,000

A thorough discussion was held of the many problems facing the journal, and it is a pleasure to record that the following recommendations were reached unanimously.

1. *MANAGEMENT*

The Association should set up a governing board, with some such title as "Board of Managers" or "Publication Board" to be responsible to the Association for the proper management of "Cancer Research." It should consist of 5 members, appointed by the President and confirmed by the Board of Directors, and be responsible to the Association through the Board of Directors.

To give continuity of policy and administration, the term of appointment should be 5 years, with one member retiring each year. The initial appointments should, therefore, be for periods of 5, 4, 3, 2, and 1 years, respectively.

This Board of Managers should select the Editor and, in consultation with the Editor, the members of the Editorial Committee. It should

select the Business Manager and be responsible for the financial and business policies of the journal. The Editor and Business Manager should attend regularly the meetings of the Board of Managers.

The Advisory Board might be continued, for a while at least, but it should have only advisory functions and no executive duties. The Chairman of the Board of Managers should attend the meetings of the Advisory Board. Membership of the Advisory Board may be changed upon the recommendation of the Board of Managers with the approval of the Board of Directors.

The division of responsibilities between an Editor and a Chairman of the Editorial Committee is not necessary. The Editor should automatically be the Chairman of the Editorial Committee.

These changes would obviate the necessity for having a Journal Committee. The Journal Committee should therefore be abolished.

2. *MAJOR POLICIES*

With responsibility and jurisdiction centered in one properly-constituted body appointed by the Association, steps should be taken to:

a) see that the journal is made current and is brought out promptly at the beginning of each month
b) see that manuscripts are processed rapidly
c) put the journal on a sound financial basis
d) increase the list of subscribers
e) take steps at once towards the assumption by the Association of ownership of its journal
f) build up an endowment fund for the journal.

3. *IMPLEMENTATION*

To clear the decks for whatever action the Board of Directors may take in these matters, the Committee has notified those on the payroll of the journal that their employment is now on a monthly basis. This will simplify whatever changes in personnel may be deemed necessary for the effective prosecution of the business of the journal.

The Committee recommends abolition of the post of *voluntary* Business Manager and the creation of a full-time, salaried position of Business Manager. His duties would be, under the direction of the Board of Managers, to:

a) increase the subscription list
b) secure paid advertising of the highest type, e.g., of books published by the leading publishers of scientific books, of laboratory apparatus, of chemicals and supplies, etc.
c) take care of records, funds, bills, correspondence, etc.
d) handle business details for the Editor and for the Board of Managers
e) should the recommended establishment of a new category of membership be approved, i.e., of "Contributing Membership," the Business Manager would contact foundations, industrial organizations, and other interested institutions and individuals who might be willing to contribute the sum of $1,000 per year or more to the maintenance of the journal.

To carry the journal through the period of transition until it becomes self-supporting, the Committee recommends that the Association apply for three grants of $5,000 each from the National Advisory Cancer Council, the Committee on Growth, and the Childs Fund, respectively, so that in the interim the expenses of publication of scientific papers may be met.

4. *OTHER ITEMS*

The Committee approved a proposed meeting with those responsible for the "Journal of the National Cancer Institute" and for "Cancer," the new journal of the American Cancer Society; Drs. Lucké and Shear are to represent "Cancer Research." This meeting has been arranged by Dr. Winternitz for February 27, 1948, at the National Academy of Sciences. "Cancer" has announced that it proposes to have a complete Abstract Section. In order to avoid unnecessary duplication, and to cut down the size of the deficit of our journal, the Committee felt that "Cancer Research" might drop its Abstract Section, for a while at least. Should the other journal's abstracts be found unsuitable for our needs, we could consider resumption of an Abstract Section in our journal if our financial position improved sufficiently.

Dr. Lucké will investigate the matter of the advisability, and the costs, of changing the present format of "Cancer Research" from an oversized page with two columns, to the standard size and format used by most scientific journals.

The Committee recommends that the Board of Directors express in an appropriate fashion the appreciation of the Association for the many services rendered by Dr. Schram to "Cancer Research" from the time of its inception to the time of her relinquishing the post of Executive Secretary of the Cancer Research Division of the Donner Foundation and, therewith, the Chairmanship of the Advisory Board of "Cancer Research."

BALDUIN LUCKÉ
ALBERT TANNENBAUM
MILDRED W. S. SCHRAM
MURRAY J. SHEAR, *Chairman*

February 21, 1948

After extended discussion it was voted to accept the report with warm thanks. Doctor Shear's motion, "That a Committee consisting of the new President and Doctors Doisy, Gardner, and Warren consult Mr. Donner concerning the transfer of the title of ownership and the nature of the support of the Donner Foundation will provide in the future; that the Board of Directors authorize the appointment by the President of a board of five managers (approved by the Board of Directors) to start work immediately with the committee just proposed; that the Board of Directors authorize the Board of Managers to adopt such of the recommendations in the Journal Committee's report as are sound and necessary; that the Board of Managers apply for funds if needed; that the Board of Directors authorize the establishment of a category of contributors as outlined in the Journal Committee's report; that all other committees and boards hitherto in-

volved in the management of *Cancer Research* be abolished," was seconded and voted favorably.

Committee on the History of the Association.—Chairman C. C. Little reported that his attempts by correspondence to obtain data upon the early history of the Association has brought no results. He suggested that the Secretary ask Dr. A. A. Thibaudeau, an earlier Secretary, for any and all old records in his possession. He also suggested that Doctor Gaylord's files may contain some relevant data. He emphasized that he would be glad to prepare a history if the facts could be gathered. Doctor Gardner mentioned that minutes of meetings after 1914 are in the American Journal of Cancer.

Committee on Memorials.—Chairman C. C. Little requested the Secretary to supply lists and instructions.

UNFINISHED BUSINESS

After brief discussion, the Board approved the present practice with respect to the make-up and handling of the program of the annual meeting of the Association.

NEW BUSINESS

The Secretary-Treasurer was authorized to drop from the rolls of the Association members in arrears three years who fail to pay their dues after being sent a registered letter stating the established policy of the Association in this regard.

It was voted that the costs of conducting the annual meeting be paid from the funds of the Association.

It was agreed that the words "upon request" be understood regarding the transfer of qualified active members to the status of emeritus members.

It was voted that the same contribution as before be made to the National Society for Medical Research.

It was voted the Secretary prepare a resolution in appreciation of Dr. Mildred W. S. Schram and send it to her at the luncheon in her honor on March 12.

It was voted that the Board of Directors express itself as being in favor of a Fifth International Cancer Research Congress.

It was voted that the new schedule of dues approved by the majority vote of the members become effective January 1, 1949. The identity of the agent for collecting the dues was left to the Board of Managers of *Cancer Research* for decision.

The meeting was now declared adjourned and immediately reconvened with Directors Aub, Bittner, Doisy, Furth, Huggins, and Taylor present. Nominations for officers for the coming year were then made: For President, Charles Huggins; for Vice-President, Joseph C. Aub; for Secretary-Treasurer, Charles W. Hooker.

It was voted to hold the next meeting of the Board at 5:45 P.M. on March 12.

The meeting was then adjourned.

JOHN J. BITTNER
Chairman, Board of Directors
CHARLES W. HOOKER
Secretary

MINUTES OF THE BUSINESS MEETING OF THE MEMBERS HELD MARCH 12, 1948

The meeting of the members of the Association was called to order at 1:45 p.m., March 12, 1948 at the Hotel Dennis, Atlantic City, New Jersey.

After waiving the reading of the minutes of the last meeting, the reports of the Treasurer and Auditor were read and accepted.

The President reported the results of the referendum on change of dues as recorded in the minutes of the meeting of the Board of Directors on March 11, 1948. It was reported that the Board had voted to adopt the change, effective January 1, 1949.

The results of the count of proxy votes for new members of the Board of Directors to serve until 1951 was reported. It was voted, "that the Secretary cast a ballot for the slate chosen."

The elections of new active members and of one Honorary Member, Dr. V. R. Khanolkar, were reported.

The nominations for officers of the Association made by the Board of Directors were read:

For President, CHARLES HUGGINS
For Vice-President, JOSEPH C. AUB
For Secretary-Treasurer, CHARLES W. HOOKER

No other nominations were made, and the slate was elected as nominated.

Dr. E. V. Cowdry presented the following resolution:

"The American Association for Cancer Research desired to record itself as actively in favor of the rapid and complete rehabilitation of the Roscoe B. Jackson Memorial Laboratory as one of the absolutely essential institutions contributing to progress in cancer research, not only within its walls but as a source of supply for research in many other institutions throughout the country."

After being seconded, the resolution was adopted.

The meeting was adjourned at 2:00 p.m.

JOHN J. BITTNER, *President*
CHARLES W. HOOKER, *Secretary*

MINUTES OF THE MEETING OF THE BOARD OF DIRECTORS HELD MARCH 12, 1948

The meeting was called to order at 5:45 p.m. at the Hotel Dennis in Atlantic City, New Jersey, following waiver of previous formal notice of the meeting signed by all Directors present and constituting a quorum. Directors Aub, Bittner, Brues, Cowdry, Doisy, Furth, Hooker, and Lucké were present, with Chairman Charles Huggins presiding.

It was voted that the President and Secretary design a "diploma" for Honorary Members.

After brief discussion it was voted that the Association take no action with respect to the World Health Organization until the relation of Doctors Parran and Scheele to the latter is clarified.

Various aspects of the Union of Biological Societies were discussed and it was decided that the President should appoint a committee to report next year.

In reporting on the Fourth International Cancer

Research Congress Dr. E. V. Cowdry, who served as its President, stated that all of the manuscripts had been sent to Doctor Maisin for publication in the *Acta* and are now in press. The 260 papers will occupy at least 1,000 pages. It turned out that although publication can be had abroad, the undertaking must be financed in the United States. An International Cancer Research Commission was organized, composed of delegates from each of the forty countries represented at the Congress; it is hoped that twenty additional countries will be represented. No member is to serve for more than three years. Doctor Cowdry expressed his desire that a member and an alternate be annually appointed from the Association, with the alternate to succeed the member. He expressed his further wish that the Association approve the appointment of Dr. W. U. Gardner as the alternate to succeed Doctor Cowdry. In reply to a question whether the Association will have to finance the travel of the member or delegate, Doctor Cowdry stated that governments abroad finance the travel of their representatives.

The following resolution was then adopted: "Resolved that the Board of Directors of the Association considers it to be its duty to make recommendations for appointments from the United States to the Commission."

It was pointed out that the By-Laws, Article I, Section 3(c), state that notices of meetings must be mailed to each member "not less than ten days nor more than forty days prior to the date of the meeting." It was agreed that forty days is too little for preparation of the program. It was accordingly voted to amend this portion of the By-Laws to read "not less than ten days prior to the date of the meeting."

The question of foreign members of the Association was again considered. After discussion it was voted "that the By-Laws as amended one year ago be affirmed."

NEW BUSINESS

It was unanimously *resolved*, "That Charles W. Hooker, Secretary-Treasurer and Charles Huggins, President, be, and each of them hereby is authorized in the name and on behalf of the Corporation to open a bank account or bank accounts with such banks, bankers and/or trust companies as they or each of them shall determine, and to deposit therein to the credit of the Corporation from time to time any and all monies and checks of the Corporation; and

Resolved, that the banks, bankers, and/or trust companies so designated as depositaries of the Corporation be, and they hereby are, severally authorized to honor and pay all checks, drafts, and other orders for the payment of money drawn upon such account or accounts (including checks, drafts, or other orders of one or both of the persons making, signing, or drawing them) made, signed or drawn by the following persons: Charles W. Hooker or Charles Huggins."

It was also "*Resolved*, that the Greenwich Savings Bank of 1356 Broadway and 985 Sixth Avenue, Borough of Manhattan, City of New York, is hereby designated as depositary of funds of this corporation and is authorized to honor drafts and orders for the payment and withdrawal of moneys therefrom made in the name of this corporation and signed by President Charles Huggins, or Secretary-Treasurer Charles W. Hooker.

"And it is Further *Resolved* that the foregoing authority shall continue until written notice of revocation of this Resolution shall be received by the Greenwich Savings Bank.

"And it is Further *Resolved* that said The Greenwich Savings Bank is authorized to accept the certificate of the Secretary of this corporation as evidence of the names and signatures of the persons at any time authorized to act pursuant to this Resolution."

It was voted that the sum of $350 be allocated for secretarial assistance.

The publication of the minutes and scientific proceedings of the meeting was authorized, the costs of publication to be paid by the Association.

It was voted "that the Program Committee set the date of the next meeting of the Association in conjunction with the meeting of the Federation of American Societies for Experimental Biology, but not in conflict with the meeting of the Pathologists."

It was voted "that a committee be appointed to handle the relations of the Association with the press at its next meeting."

The Chairman then proposed the following standing committees:

Program Committee.—J. C. AUB, *Chairman;* H. L. TAYLOR, JR., F. W. STEWART.

Nominating Committee.—C. W. HOOKER, *Chairman;* W. E. HESTON, I. T. NATHANSON.

Membership Committee.—JACOB FURTH, *Chairman;* A. M. BRUES, G. H. TWOMBLY.

Cancer Research, its Organization and Support.—SHIELDS WARREN, *Chairman;* G. M. SMITH.

The Board approved the proposed Committees

The Chairman proposed as a *Committee on Local Sections:* E. V. COWDRY, *Chairman;* M. B. SHIMKIN, T. F. DOUGHERTY. The Board approved these selections.

The Chairman, with the approval of the Board, appointed as members of the Board of Managers of *Cancer Research:*

BALDUIN LUCKÉ, 5 years
PAUL E. STEINER, 4 years
ALBERT TANNENBAUM, 3 years
E. W. SHRIGLEY, 2 years
C. C. LITTLE, 1 year

The meeting was adjourned at 6:55 P.M.

CHARLES HUGGINS
Chairman, Board of Directors
CHARLES W. HOOKER
Secretary

New Books

Cancer. Tome II. Radiations, Virus, Environment. By J. MAISIN, Professeur à l'Université de Louvain, Directeur de l'Institut du Cancer. Tournai-Paris: Casterman, 1949. Pp. 308. 120 francs.

This second review volume on cancer by Professor Maisin contains three chapters on the subjects stated in the subtitle. In the chapter on radiations are discussed the carcinogenic action of infra-red, visible light, and x-rays and gamma-rays as well as the importance of the different routes of exposure to radioactive substances namely, injection, ingestion, and inhalation. The chapter on viruses contains also information on parasites and other living agents. The final chapter deals with divers carcinogens, the role of vitamins, enzymes, and other influences in producing cancer. The views are stimulating and, on the whole, conventional. Although the book was published in 1949, it is dated 1946. The bibliography is extensive but unfortunately deficient in some important pertinent titles since 1941. The book is especially valuable for integrating much information and many ideas from related sciences into the cancer field.

Krebsmetastasen. By DR. MED. HANS E. WALTHER, Leitender Arzt der rontgenabteilung des Schwesternhauses vom Roten Kreuz, Zurich. Verlag—Basel: Benno Schwabe & Co., 1948. Pp. 560. Ganzleinen Fr. 60.

The time, place, and extent of tumor metastases are important in diagnosis, treatment, and prognosis. The general behavior patterns in metastasis are known for the common tumors, although much exact work remains to be done. The present volume is a major contribution to this problem. It was written by a roentgenologist whose daily need for more precise knowledge stimulated the work. It is based on 3433 tumor autopsies meticulously studied. The book is divided into general and special sections. The former deals with the basic rules in metastasis; the latter with specific organs and systems. The numerical data are presented in 173 tables. Nearly 300 figures illustrate the principal points by diagram, photograph, roentgenogram, and photomicrograph. References with complete title are given at the point of citation. The author has introduced evidence on many debated points. For example, he denies the importance of retrograde metastasis and insists that the dissemination of tumors by way of the blood and lymph follows anatomic and physiological laws of circulation.

This book was intended primarily for the use of the surgeon, radiologist, and pathologist to enable them to locate the primary tumor when metastases are discovered, or to give the most likely site of metastasis from a known primary tumor. Its value, however, goes far beyond that point. Nearly everybody working in cancer should find it useful. Here is presented one important aspect of the natural life history of human tumors. The laboratory worker can quickly compare the behavior of his experimental tumor with the human counterpart. The data are sure to be widely quoted. The book should be found useful for quick reference in every cancer library.

Announcement

Dr. Harold P. Rusch will become Editor-in-Chief of *Cancer Research* beginning with Volume 10, January, 1950. All manuscripts submitted for publication should be sent to him care of McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wisconsin, effective immediately.